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Physics of cell adhesion failure and human diseases

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Abstract

Emergent phenomena in living systems, including your ability to read these lines, do not obviously follow as a consequence of the fundamental laws of physics. Understanding the physics of living systems clearly falls outside the conventional boundaries of scientific disciplines and requires a collaborative, multidisciplinary approach. Here I will discuss how theoretical and computational techniques from statistical physics can be used to make progress in explaining the physical mechanisms that underlie complex biological phenomena, including major diseases. In the specific cases of macular degeneration and cancer that we have studied recently, we find that the breakdown of the mechanical stability in the local tissue structure caused by weakening of the cell-cell adhesion plays a key role in the initiation and progression of the disease. This finding can help in the development of new therapies that would prevent or halt the initiation and progression of these diseases.

1. Introduction

Life in all of its forms, including your ability to read these lines, does not follow from fundamental laws of physics. This fact precludes direct application of physical laws to biological systems. In his 1944 monograph called "What is Life?" Erwin Schrödinger (1944) proposed that life had two secrets: the passage of an encoded molecule

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from parents to offspring that is responsible for heritable characteristics, and the spontaneous emergence of order in living systems. The first idea has culminated in the unprecedented progress made in genetics and molecular biophysics. On the other hand, still little is known about the origin of emergent phenomena in *living systems*, even though some progress has been made in elucidating collective behavior and self-organization in certain condensed matter and quantum systems.

There are subtle distinctions that separate many toy models exhibiting emergent behavior in biologically inspired models from predictive understanding of emergent phenomena in living systems, including the pathogenesis of human diseases. This lack of progress is clearly evident, for example, in the fact that the physical mechanisms of only an anecdotally small number of catastrophic ailments have been discovered.

In this paper I will review the results of some recent work, Shirinifard et al. (2011), Gryn and Family (2014), we have done using computer simulations of realistic models of biological systems to investigate the initiation, progression and spreading of two types of human diseases: (1) Age-Related Macular Degeneration, and (2) Cancer Tumor Growth and Invasion. The main question we address here is what is the role of cell-cell adhesion failure in the transition from a healthy organ to one that promotes initiation and spreading of a disease? Organs in living systems are made up of tissues that communicate with other cell types via cell–cell interactions at the cell surface. Our main finding is that pathogenic processes cannot be attributed alone to genetic changes in a single cell, but are the result of the complex interplay between the failure of cellular regulation mechanisms and the presence of a permissive microenvironment. In fact our results show that in the cases that we have studied the biological systems become vulnerable to growth and spreading of diseases once the mechanical and physical stability of a tissue is compromised due to weakening of cell adhesion. This suggests that therapies based on controlling or eliminating adhesion failure could be effective approach for preventing and halting the initiation and progression of certain diseases.

2. Simulating Biological Systems

A computational approach is perhaps the most effective way to deal with the multilevel complexity typical of biological systems. Such an approach allows us to simplify the biological problem by providing both a concise description of its essential features and the possibility of incorporating the relevant mechanisms and parameters. A significant advantage of a computational approach is that it provides a controlled way of determining the consequences of different experimental conditions and serves as a guide for future experiments

Recent advances in condensed matter and statistical physics have been achieved by modeling disparate types of materials as collections of large number of identical interacting elements. When viewed as a unique state of matter, biological organs and tissues can also be considered as a collection of interacting and inter-communicating cells representing the fundamental elements of the system at a mesoscopic scale. In this type of description, cells are represented by one or more set of discrete units with rules describing their movements and interactions. One of the most effective approaches of this type is the Cellular Potts Model, Glazier and Graner (1993), Swat et al. (2012), (also known as the Glazier-Graner-Hogeweg model, Balter et al. (2009)) in which each element can be represented by a collection of spatial units that capture the biophysical properties of individual cells, such as shape, movement or adhesion, and their interactions with the neighboring cells. In this type of approach, the cell-scale elements obey a set of prescribed rules, that may depend on their type and on the signals they receive from the neighbors and from the environment. CPM has been successfully used to model many problems in biological and biomedical field, including morphogenesis, cell sorting, angiogenesis, wound healing, and tumor vasculogeneis, as well as non-biological applications, including simulations of foam drainage and foam rheology (see Swat et al. (2012), Balter et al. (2009) for references to various applications).

The Cellular Potts Model (CPM) is a generalization of the Q-state Potts Model from statistical mechanics and shares with it the idea of modeling dynamics using energy minimization under random fluctuations. The details of the CPM approach can be found in several publications (see Swat et al. (2012), Balter et al. (2009) and references therein) as well as at the CompuCell3D website (http://www.compucell3d.org), where extensive tutorials and guides about CPM are available. Cells in the CPM are represented as spatially extended but internally structureless objects with complex shapes. Cell behaviors and interactions are described in terms of effective energies. On the lattice, each site or pixel is associated with a single spin. A collection of pixels with the same spin represents a cell. The

change in shape of the cell and/or cell motion happens through mutation of spins of individual pixels. Any physical process that can be described in terms of an internal energy term can be incorporated in the simulation by adding that energy term to the effective energy. At each time step a lattice site belonging to a particular cell is randomly selected and proposed to copy its spin value into a randomly selected nearest-neighbor site in the neighboring cell. The probability for this exchange depends on the energy cost between the initial and the final configuration. Since the probability must remain invariant under a constant shift in the energy scale, the only function that satisfies this condition is the exponential function. Thus, a Metropolis type algorithm is used for spin updates that are accepted with Boltzmann-like probability.

Cellular adhesion is essential in maintaining multicellular structure, because it plays a key role in the movement and the mechanical stability of biological tissues. Cell adhesion is the binding of a cell to a surface or substrate, the extracellular matrix, or another cell. Adhesion occurs from the action of proteins called cell adhesion molecules, such as *selectins*, *integrins*, and *cadherins*. In addition, cellular adhesion can link the cytoplasm of cells and can be involved in signal transduction. Consequently, one of the key terms in the system effective energy is the adhesion energy (Glazier and Graner (1993)). The adhesion energy is based on the *Differential Adhesion Hypothesis*, first proposed in 1960's by Steinberg and others, Foty and Steinberg (2005), which asserts that cellular movement is controlled by the rearrangement of cells to a more thermodynamically stable structure. This is achieved by maximizing the amount of energy that is utilized adhering the cells together, which decreases the free energy available in the system. As cells with similar strengths of surface adhesion bond to one another, bonding energy in the overall system increases, and interfacial free energy decreases causing the system to be more thermodynamically stable. As I will discuss below, our results show that cell adhesion failure is a key mechanism in the pathogenesis of two specific diseases that we have studied.

3. Age-Related Macular Degeneration (AMD)

Age-Related Macular Degeneration (AMD) is the leading cause of blindness in the elderly and is a looming epidemic in our aging society, Holz et al. (2011). Currently, no effective therapies are available for prevention or halting of this debilitating disease. Choroidal neovascularization (CNV), Grossniklaus (2004), is the pathogenic mechanism that leads to more severe form of AMD. In CNV, new blood vessels from the choriocapillaries initially penetrate the Bruch's membrane (BrM) and then spread into the retina layers and eventually affect the photoreceptors, causing irreversible vision loss. Our results question the validity of the current hypotheses regarding the causes of CNV.

CNV involves the interaction of two fundamental structures of the eye, the retina and the choroid. The outer retina is a layered structure that is situated between the photoreceptors and the choroid. The choroid lies in the posterior of the eye and consists of the dense choriocapillaris that bring oxygen and nutrients to the eye and dispose of chemicals and other waste products. The retinal pigment epithelium (RPE) is a monolayer of pigmented epithelial cells that is situated between the photoreceptors and the choroid. The RPE plays numerous roles in maintenance of the retina. Between the RPE and the choroid lies Bruch's membrane (BrM), a strong, multi-layered collageneous membrane that separates the RPE from the choriocapillaris. The inner part of the retina, adjacent to the vitreous humor, includes the retinal vasculature and layers of neural cells. The outer retina, adjacent to the RPE, includes the rod and cone photoreceptors.

Multiple hypotheses have been proposed to explain CNV initiation, growth and patterning (for comprehensive reviews, see Spade et al. (2003), Zarbin et al. (2004)). These hypotheses are divided into two major groups based on their proposed mechanism of action: (1) VEGF overexpression, and (2) irregularities in BrM. A key factor that had not been considered is the role of interactions among the different types of cells and the possibility that their impairment could have an effect on CNV. To better understand the mechanism for CNV, we have considered the role and the correlations of adhesion failure in the initiation and progression of CNV.

We have used CPM and developed a three dimensional model, Shirinifard et al. (2012), that allows us to probe the effect of specific mechanisms without confounding crosstalk and to study the effects of multiple mechanisms acting simultaneously or sequentially. This approach allowed us to explore many more combinations of bio-mechanistic hypotheses and parameter choices than we could in experiments. We simulated different adhesion scenarios by assigning one of three levels: normal, moderately impaired, and severely impaired to each of the five

key adhesion parameters, Shirinifard et al. (2012). For each adhesion scenario we simulated our retina both in the absence and presence of a tip cell for one simulated year.

We have developed, Shirinifard et al. (2012), a morphometric quantification and classification algorithm to classify CNV patterns. Our classification scheme is compatible with current histological classifications and can be applied to images of appropriately labeled sections and 3D images of the retina.

We find that previously neglected RPE-BrM, RPE-RPE and RPE-POS adhesion failures suffice to determine the loci and progression of choroidal neovascularization. Our simulations confirm all clinically known patterns of CNV. But, while most studies hypothesize that CNV primarily results from the effects of excess VEGF or holes in BrM, we find that CNV results from combinations of impairment of RPE-RPE junctional adhesion, adhesion failure in RPE-BrM complex, and adhesion of the RPE to the photoreceptor outer segments (RPE-POS adhesion). These findings suggest that defects in adhesion dominate CNV initiation and progression.

4. Cancer Tumor Growth and Invasion

Normal cells in a tissue survive within well-regulated and mechanically stable environmental conditions and are subject to microenvironmental control. On the other hand, tumor cells must overcome a host of hostile microenvironment conditions, including hypoxia, growth factor deprivation, and loss of adhesion to the ECM. Therefore, understanding how cancer tumors grow is made difficult by the complexity that arises from the interplay of several factors including cancer cell heterogeneity, tumor cell interactions within the tumor and with the local extra cellular matrix (ECM) and stochastic mutations and adaptations during progression. The question we address here is: how do tumor cells overcome these obstacles and are able to invade the ECM? Specifically, we have concentrated on the role of microenvironment modifications and the resulting weakening of ECM adhesion on cancer tumor growth and invasion.

Invasion is basically a three-step process involving changes in tumor cell adhesion, degradation of the ECM and finally the ability of the tumor cells to migrate in the modified ECM environment. This is consistent with the idea that the major function of the ECM is to provide a scaffold to support the organization of cells into specific tissues. Under normal conditions, the ECM provides a physical barrier against invasion and metastasis. However, the ECM is linked strongly, through cell adhesion processes, to the tumor cells. Modifications to this interaction that degrade cell adhesion can facilitate not only tumorigenesis, but also the process of tumor invasion.

We have developed a three-dimensional model using the Cellular Potts Model and have studied the dynamics of the growth of a cancer tumor cell in a healthy ECM microenvironment. We have used the parameters that have been used in previous models of tumor growth and angiogenesis, Swat (2012), Balter (2009), and have exclusively concentrated on the role of adhesion failure in tumor growth. To invade the extracellular matrix environment, a cell has to adhere to its local environment in order to pull itself through the matrix. Clinical and experimental studies have long shown that cancer cells modify the local environment through various mechanisms such as changes in the local H^+ concentration, Estrella (2013), and release of enzymes. When the local matrix is too dense, the cell has to degrade the matrix in order to get through, but when it does not have sufficient friction, the cell produces proteins to locally increase its traction.

Our results show that changes to the microenvironment and weakening of cell-cell adhesion, due to such processes as chronic inflammation or upregulation of enzymes, strongly correlate with cancer tumor survival, growth and invasion. A heterogeneous local environment enables tumor cells to migrate more easily and adapt to other unfavorable conditions, such as hypoxia by migrating along the gradients of oxygen and nutrient concentrations, Estrella (2013). By studying the velocity correlation among motile cells, we have found that collective motion of cells is also enhanced with cell adhesion weakening. Enhanced collective behavior is perhaps an additional mechanism that promotes cancer invasion. Our results support the longstanding hypothesis, Mantovani (2013), that persistent inflammatory conditions promote tumor growth by continually upregulating enzymes that weaken the adhesion properties of the ECM. The results also provide support for another finding that the pH of solid tumors is acidic and the diffusion of H^+ into the tumor microenvironment leads to remodeling of the microenvironment and therefore cancer invasion, Estrella (2013). This is partly due to the ability of cancer cells to adapt and thrive in acidic environments.

5. Conclusions

In this brief report I have discussed the results of studying the initiation and progression of two different types of diseases, age-related macular degeneration and cancer tumor growth and invasion. The goal of these studies was very specific: To determine the possible role of cell adhesion weakening on the initiation and progression of these diseases. Cellular Potts Model is an effective approach for such a study, as it provides a mathematical and computational environment that allows for realistic modeling of biological systems from the cellular to the macroscopic sizes. The main finding of these studies is that adhesion failure can be a key factor in the development of diseases. This finding supports, for example, the hypothesis that inflammation, which causes mechanical degradation of the local tissue by weakening of the cell adhesion, plays a key role in promoting certain diseases, including cancer tumor growth and invasion.

Acknowledgements

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