Part I

Theory

1 Introduction

In this book we describe recent efforts to model tumor growth and invasion using an interdisciplinary approach that integrates mathematical and computational models of cancer with laboratory experiments and clinical data. The aim of these efforts has been to provide insight into the root causes of solid tumor invasion and metastasis, to aid in the understanding of experimental and clinical observations, and to help design new, targeted, experiments and treatment strategies. The ultimate goal is for modeling and simulation to aid in the development of individualized therapy protocols that minimize patient suffering while maximizing treatment effectiveness. In order to achieve this objective, mathematical and computational models are needed that quantify the links of three-dimensional tumor-tissue architecture with the growth, invasion, and underlying micro-scale cellular and environmental characteristics. This approach requires a multiscale modeling framework that is capable of linking the molecular and cell scales directly to the patient data.

There are many ways to evaluate the progression of these efforts. Here we follow the major stages that progressively incorporate the complexity of the tumor environment: (i) modeling of avascular tumors *in vitro* and *in silico* to assess stages of tumor growth; (ii) interactions between a tumor and its *in vivo* microenvironment; (iii) modeling of vascularized tumors *in silico* to assess angiogenesis and vascular growth; (iv) modeling of vascularized tumors *in vivo* and *in silico* to assess tumor progression in the body. We describe in detail biologically founded approaches that employ multiscale mathematical models of tumor progression in two and three dimensions. This enables the study of how molecular-scale phenomena regulating cell-proliferation, migration, and adhesion forces, including those associated with the genetic evolution from lower- to higher-grade tumors, may generate, in a predictable and quantifiable way, heterogeneous cell activity and oxygen and nutrient demand across the tumor mass, thus determining its morphology and degree of invasiveness.

Through the years numerous mathematical models have been developed to study the progression of cancer (e.g., see the reviews by Adam [5], Chaplain [120], Bellomo and Preziosi [61], Moreira and Deutsch [475], Bellomo *et al.* [59], Swanson *et al.* [649], Araujo and McElwain [34], Mantzaris *et al.* [452], Friedman [236], Ribba *et al.* [566], Quaranta *et al.* [553], Hatzikirou *et al.* [307], Nagy [490], Byrne *et al.* [96], Fasano *et al.* [209], van Leeuwen *et al.* [668], Roose *et al.* [573], Graziano and Preziosi [291], Friedman *et al.* [237], Sanga *et al.* [584], Deisboeck *et al.* [169], Anderson and Quaranta [29], Bellomo *et al.* [60], Cristini *et al.* [146], and Lowengrub *et al.*

[425]). Most models fall into two broad categories based on how the tumor tissue is represented: discrete cell-based models and continuum models. Although the continuum and discrete approaches have each provided important insight into cancer-related processes occurring at particular length and time scales, the complexity of cancer and the interactions between the cell- and tissue-level scales may be elucidated further by means of a multiscale (hybrid) approach that uses both continuum and discrete representations of tumor cells and components of the tumor microenvironment. Thereby biological phenomena from the molecular and cellular scales are coupled to the tumor scale (e.g., the recent work by Kim *et al*. [382], Bearer *et al*. [57] and Lowengrub *et al*. [425]). Such an approach can, for example, capture transitions from collective to individual behavior and combine the best features of continuum and discrete models.

Continuum tumor models are based on reaction–diffusion equations describing the tumor cell density (e.g., [22, 532, 680]), the extracellular matrix (ECM), matrix degrading enzymes (MDEs) (e.g., [82, 83, 123, 316]), and concentrations of cell substrates such as glucose, oxygen, and growth factors and inhibitors (e.g., [73, 118, 121, 303, 315, 442, 495]). Classical work [292, 293] used ordinary differential equations to model tumors as a homogeneous population, as well as partial differential equation models confined to a spherical geometry. In the case of avascular tumors, growth has been modeled as a function of cell substrate concentration, usually oxygen. More recent work has incorporated cell movement through diffusion (e.g., [121, 607, 608]), convection (e.g., [101, 157, 532, 679, 682]), and chemotaxis or haptotaxis (e.g., [453, 532, 607]). Cell proliferation, death, and pressure have also been considered (e.g., [21, 40, 59, 78–80, 92–95, 100, 101, 103, 104, 149, 164, 220, 222, 258, 262, 318, 335–338, 352, 399, 413, 429, 532, 536, 574, 589, 632, 636, 657, 673, 681]). Linear and weakly nonlinear analyses have been performed to assess the stability of spherical tumors to asymmetric perturbations (e.g., [34, 95–97, 99, 100, 104, 121, 149, 273, 416]) in order to characterize the degree of aggression. Various interactions with the microenvironment, such as nutrient- or stress-induced limitations to growth, have also been studied (e.g., [18, 20, 22, 35, 37, 38, 352, 574]). The models may account for observations of the stronger cell–cell interactions (cell–cell adhesion and communications), the high polarity, and the strong pulling forces exchanged by cells and the ECM [127–129, 235]. Extracellular matrix reorganization by tumor cells [235] has been incorporated, and various degrees of dependence of the cells on signals from the matrix have been modeled. The models are typically single-species (e.g., single-phase tumors), treating the tumor or, more generally, biological tissues as fluid (e.g., [80, 81, 97, 99, 100, 103, 119, 241, 293]), elastic or hyperelastic (e.g., [17, 20, 35, 248, 249, 257, 328, 352, 462, 604, 673]), poroelastic (e.g., [574]), viscoelastic (e.g., [46, 382]), or elasto-viscoplastic (e.g., [23]). More recently, multiphase models (e.g. using mixture theory) have been developed to simulate multiple solid-cell species and extra- or intracellular liquids (see below). Theoretical nonlinear analyses of the various single-phase tumor models mentioned above have also been performed (e.g., [55, 86, 150–160, 176, 209, 236, 238–240, 242, 243, 490, 520, 608, 652, 653, 675, 701, 706, 707, 723, 724]).

Building upon the formulation of classical models [100, 101, 293, 460], a breakthrough nonlinear simulation of a continuum tumor model was provided by Cristini *et al*. in 2003 [149], who studied solid tumor growth in the nonlinear regime using boundary-integral simulations in two dimensions to explore complex morphologies. This work demonstrated that non-necrotic tumor evolution could be described by a reduced set of two parameters that characterize families of solutions. One parameter describes the relative rate of mitosis to the relaxation mechanisms (cell mobility and cell–cell adhesion). The other describes the balance between apoptosis (programmed cell death) and mitosis. Both parameters also include the effect of vascularization. The results revealed that tumor growth can be divided into three regimes, associated with increasing degrees of vascularization: low (diffusion-dominated), moderate, and high. Critical conditions exist for which the tumor evolves to nontrivial dormant states or grows self-similarly (i.e., in a shape invariant way). Away from these critical conditions evolution may be unstable, leading to invasive fingering into the external tissues and to topological transitions such as tumor breakup and reconnection. This work identified for the first time the concept of tumor "diffusional instability" in the low vascularization regime as a mechanism for invasion. While previous work [97, 99, 100] had demonstrated that steady-state avascular symmetric tumors could be unstable, the results of Cristini *et al*. [149] showed that instability during growth can allow tumors to grow indefinitely, bypassing the symmetric steady state. This idea has since been studied in a variety of different configurations using a number of different models. Interestingly, the shape of highly vascularized tumors was predicted to remain compact and without invasive fingering, even while growing unboundedly. This suggests that the invasive growth of highly vascularized tumors is associated with vascular anisotropy and other inhomogeneties in the microenvironment (e.g. cell-substrate inhomogeneities, elastic anisotropy). The self-similar behavior described above leads to the possibility of controlling tumor shape control and of the release of tumor angiogenic factors by restricting the tumor volume-to-surface-area ratio.

Expanding on the idea of "diffusional instability," subsequent work [116, 145, 230, 436, 437, 620, 722] has developed the hypothesis that, through heterogeneous cell proliferation and migration, microenvironmental substrate gradients, e.g., of cell nutrients or the extracellular matrix, may drive tumor invasion through morphological instability with separation of cell clusters from the tumor edge and infiltration into surrounding normal tissue. Tumor morphology would be determined by the competition between heterogeneous cell proliferation caused by spatial diffusion gradients, driving shape instability, and invasive tumor morphologies, and stabilizing mechanical forces, e.g., cell–cell and cell–matrix adhesion. Following these ideas, the stability of avascular tumor growth has also been investigated in discrete models [25, 273, 538].

To investigate further the stability of avascular tumors, parameter-based statistics providing input to the mathematical model were obtained from *in vitro* glioblastoma tumors [230]. Employing a linear stability analysis of the model from Cristini *et al*. [149], these results predicted that tumor spheroid morphology would be marginally stable. In agreement with this prediction, unbounded growth of the tumor mass and invasion of the environment were observed *in vitro*. The mechanism of tumor invasion was characterized

as recursive sub-spheroid component development (i.e., the formation of "buds") at the tumor viable rim and separation from the parent spheroid. Computer simulations of the mathematical model closely resembled the morphologies and spatial arrangement of tumor cells from the *in vitro* model. Simulations and *in vitro* experiments provided further evidence [145] that morphological instability could be suppressed *in vivo* by spatially homogeneous oxygen and nutrient supply because normoxic conditions act both by decreasing gradients and increasing cell adhesion and, therefore, the mechanical forces that maintain a well-defined tumor boundary. Taking into account the effect of the microenvironment, it was also found that tumor morphological stability could be enhanced by improving the nutrient supply [437].

Recently, multiphase (mixture) models have been developed that are capable of describing detailed interactions between multiple solid cell species and extra- or intracellular liquids (see Chapter 5 for references). Vascular tumor growth has been studied in three dimensions [57, 229], and the simulation results compare well with clinical tumor data. In particular, in [57] a biologically founded multiphase model was applied to identify and quantify tumor biologic and molecular properties relating to clinical and morphological phenotype, and to demonstrate that tumor growth and invasion are predictable processes governed by biophysical laws and regulated by heterogeneity in phenotypic, genotypic, and microenvironmental parameters. This heterogeneity drives the migration and proliferation of more aggressive clones up the cell substrate gradients within and beyond the central tumor mass, while often also inducing loss of cell adhesion. The models predict that this process triggers a gross morphologic instability that leads to tumor invasion via individual cells, cell chains, strands, or detached clusters infiltrating into adjacent tissue and producing the typical morphologic patterns seen, e.g., in the histopathology of brain cancers such as glioblastoma multiforme. The model further predicts that the different morphologies of infiltration correspond to different stages of tumor progression regulated by heterogeneity.

This mathematical and computer modeling provides evidence that tumor morphogenesis *in vivo* may be a function of marginally stable environmental conditions caused by spatial variations in cell nutrients, oxygen, and growth factors. A properly working tumor microvasculature could help maintain compact non-infiltrating tumor morphologies by means of minimizing the oxygen and nutrient gradients. Controlling the environmental conditions by decreasing spatial gradients may benefit treatment outcomes whereas current treatments, and especially anti-angiogenic therapy, may trigger microenvironmental heterogeneity (e.g., local hypoxia), thus causing invasive instability. Indeed, the mathematical models show that the parameters that control the tumor mass shape also control its ability to invade [149, 230]. Thus, tumor morphology may serve as a predictor of invasiveness and treatment prognosis.

The theoretical models also provide an explanation for the highly variable outcome of anti-angiogenic therapy in multiple clinical trials [145]. Anti-angiogenic therapy may promote morphological instability, leading to invasive patterns even under conditions in which the overall tumor mass shrinks. Thus, therapeutic strategies focused solely on the reduction of vascular density may paradoxically increase invasive behavior. Anti-angiogenic strategies may be more consistently successful when aimed at "normalizing"

the vasculature [342] and when combined with therapies that increase cell adhesion [528], so that morphological instability is suppressed and compact non-invasive tumor morphologies are enforced. This could be done, for example, through anti-invasive therapy [468, 476]. Further, by quantifying the link between the tumor boundary morphology and the invasive phenotype, multiscale mathematical modeling provides a quantitative tool for the study of tumor progression and diagnostic and prognostic applications. This establishes a framework for monitoring system perturbation towards the development of therapeutic strategies and obtaining correlations to clinical outcome for prognosis.

The outline of this book is as follows. In Part I, we focus on the theory and the numerics. In Chapter 2, we review basic cancer biology as a background to the modeling. In Chapter 3, we present continuum modeling and the incorporation of biologically relevant parameter values into multiscale models of tumor growth and invasion. We describe a basic model founded on classical work and then expand it to include vascularization. In Chapter 4, we evaluate the theory of stability, including different regimes of growth and linear analyses. We then consider in Chapter 5 multiphase modeling to simulate multiple cell species and include the effects from tumor cell chemotaxis as well as tumor-induced vascularization in three dimensions. Chapter 6 presents the modeling of discrete cells by evaluating an agent-based cell model with applications to cancer. Chapter 7 explores the modeling of tumor invasion by using a hybrid continuum–discrete multiscale framework. Chapter 8 introduces the numerical schemes used to solve the model equations.

In Part II, we look at specific case studies. Chapter 9 describes the multidisciplinary modeling of tumor growth and invasion through the incorporation of experimental and clinical observations into the parameters of the tumor model. Chapter 10 considers the application of agent-based modeling to breast cancer. The Conclusion following Chapter 10 introduces patient-specific modeling with a prototype of the multiscale modeling framework by calibrating the molecular or cell scale directly to patient data and upscaling to calibrate the continuum scale.

2 Biological background[1]

With P. Macklin

In this chapter, we present some of the key biological concepts necessary to motivate, develop, and understand the tumor models introduced in this book. We introduce the molecular and cellular biology of noncancerous tissue (Section 2.1) and then discuss how this biology is altered during cancer progression (Section 2.2). The discussion may be more detailed in some areas than is necessary for the models that we present; the intention is to offer a sample of the rich world of molecular and cellular biology, helping the reader to consider how these and other details may need to be incorporated in the work of cancer modeling. For greater depth on any of the topics, refer to such excellent texts as [12] for molecular and cellular biology and [384] for cancer cell biology.

2.1 Key molecular and cellular biology

We focus upon the molecular and cellular biology of epithelial cells, the stroma, and the mesenchymal cells that create and maintain the stroma (Section 2.1.1). Specific and often anisotropic adhesive forces help to maintain tissue architecture (Section 2.1.2). Epithelial and stromal cells have the same basic subcellular structure (Section 2.1.3) and share much in common. They progress through a cell cycle when preparing to divide, can control their entry into and exit from the cycle, and can self-terminate (apoptose) when they detect irreparable DNA errors or other damage (Section 2.1.4). Their behavior is governed by a signaling network that integrates genetic and proteomic information with extracellular signals received through membrane-bound receptors (Section 2.1.5). Sometimes cells respond to signaling events by moving within the stroma or along the basement membrane (Section 2.1.6). In pathologic conditions leading to hypoxia, cells can respond through a variety of mechanisms or can succumb to necrosis; in some cases, the necrotic cellular debris becomes calcified (Section 2.1.7).

2.1.1 Tissue microarchitecture and maintenance

The *epithelium* is composed of sheets of tightly adhered epithelial cells that cover organ surfaces and often perform specialized functions. The epithelium is supported by the *stroma*, a loose connective tissue. The main component of the stroma is the *extracellular*

[1] This introduction to cancer biology updates and expands the original exposition in [431].

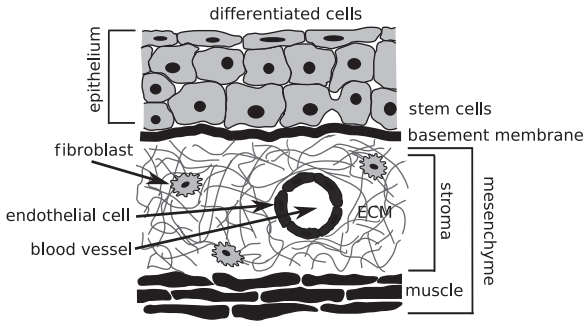

Figure 2.1 Typical tissue structure showing epithelium separated from the stroma by a basement membrane.

matrix (ECM), a scaffolding of fibers (collagen, elastin, fibronectin, etc.) embedded in a mixture of water and glycoproteins. The ECM is secreted and maintained by *stromal cells*, specialized mesenchymal cells that can freely move within the stroma as they maintain the tissue; fibroblasts are the primary stromal cells in loose connective tissue (epithelial stroma). The stroma is interlaced by blood vessels, nerves, and lymphatic vessels, and it may rest on an additional layer of muscle or bone, depending upon the location. A thin, semi-permeable *basement membrane* (BM, a specialized type of ECM) separates the epithelium from the stroma. See Figure 2.1.

This complex tissue structure is maintained by careful regulation of the cell population and a specific balance of adhesive forces. These processes are often tied together through cell signaling. For further information on tissue and organ structure, see [219] and [12] and the references therein.

Population dynamics

Each cell type population must be regulated by balancing proliferation and apoptosis. When a differentiated cell dies, a *somatic stem cell* may divide either symmetrically into two new stem cells or asymmetrically into a stem cell and a *progenitor cell*. The progenitor cell either further divides or terminally differentiates into a specific cell type and then migrates or is pushed to the correct position and assumes its function. This process is tightly regulated by intercellular communication via biochemical signals (growth factors) and mechanics; stromal cells help to maintain this signaling environment [423, 493, 725]. Each cell's response to the microenvironment is governed by surface receptors that interact with an internal signaling network. We note that stem-cell dynamics are not fully understood; see the excellent overviews in [71, 725].

Epithelial cell polarity and adhesion

Epithelium can be broadly classified as *simple* or *stratified* on the basis of its cell arrangement. In simple epithelium, cells are arranged in a single layer along the basement membrane. The cells are *polarized*, with a well-defined base adhering to the BM and an apex exposed to the *lumen* (e.g., a cavity in an organ); the apical side of the cell is often used to release secretory products. The epithelial cells adhere tightly to one another along their nonapical, nonbasal, sides. See Figure 2.2, left. In stratified epithelium, a

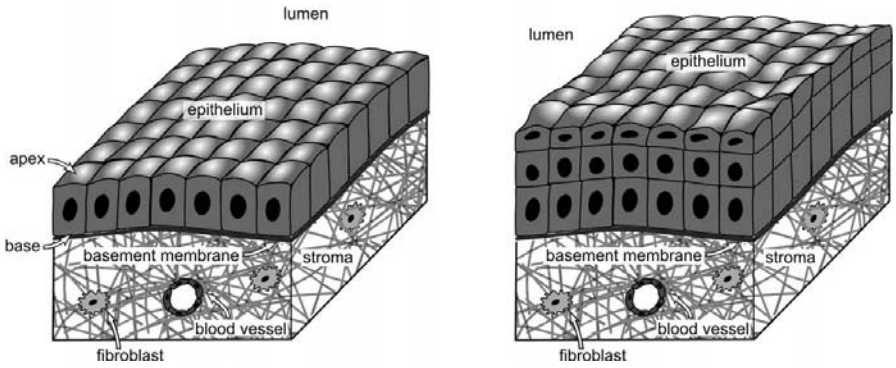

Figure 2.2 Simple (left) and stratified (right) cuboidal epithelium.

single cell layer adheres to the BM (as in simple epithelium), with additional layers above. The cells in the upper layers adhere to the layers above and below them and tend to be flattened. See Figure 2.2, right. Overall, the careful orchestration of cell–BM and cell–cell adhesion helps determine the tissue geometry [301, 350, 685]. In fact, heterogeneities in the balance of cell–cell and cell–BM adhesion can lead to epithelium invagination [400], folding [672], and other nontrivial geometries [633]. The molecular mechanisms of adhesion are further explored in Section 2.1.2. More information on epithelial cell polarization can be found in standard biology texts, such as [12].

Interaction between cell adhesion and population dynamics

Cell adhesion and population dynamics are, in fact, linked to one another. Epithelial cell cycle progression and proliferation are controlled in part by cell–cell adhesion: when an epithelial cell is in (adhesive) contact with many neighbors, its cell cycle and proliferation are suppressed. This helps to maintain the epithelial cell population by reducing proliferation when the epithelium is fully populated, and by increasing proliferation near gaps in the epithelium (e.g., due to apoptosis) [142, 301, 685]. Hence, cell–cell contact-dependent proliferation helps to prevent overproliferation. This theme is discussed further in Section 2.1.5.

Cell populations are also controlled by contact with the extracellular matrix and basement membrane. Polarized epithelial cells often become apoptotic after losing adhesive contact with the BM [245, 276, 330, 640, 677]; this specialized type of apoptosis, termed *anoikis*, helps prevent overproliferation of unattached cells into the lumen [162]. The ECM also plays a major role in regulating stromal cells [276]. For example, ECM-bound proteoglycans control the proliferation, differentiation, and apoptosis of bone marrow stromal cells [68] and integrin ligands in the ECM regulate endometrial stromal cells [598].

2.1.2 Cellular adhesion and cell sorting

Adhesion is essential to multicellular arrangement and motility: cell–cell, cell–ECM, and cell–BM adhesion are responsible for maintaining the tissue arrangement, while cell–BM and cell–ECM adhesion are essential for traction during motility.

Adhesion

Cells can exhibit both homophilic and heterophilic adhesion. In *homophilic* adhesion, adhesion receptor molecules on the cell surface bond to identical ligands (a receptor's "target" molecules) either on neighboring cells (in cell–cell adhesion) or in the microenvironment (in cell–ECM or cell–BM adhesion). This is the mode of E-cadherin-mediated cell–cell adhesion in epithelial cells, including carcinoma [521]. In *heterophilic* adhesion, surface adhesion molecules of one type bond to unlike ligand molecules in the extracellular matrix, on the basement membrane, or on neighboring cells. Cell–ECM and cell–BM adhesion are heterophilic between integrin molecules on the cell surface and ligands such as laminin and fibronectin in the microenvironment [90]. Heterophilic cell–cell adhesion is also observed, for example in T-cell lymphocytes via immunoglobulin–integrin bonds [427, 630, 654].

Cell adhesion and cell sorting

While epithelial cell–cell adhesion is generally homophilic and mediated by E-cadherin, other cadherins complicate the picture. For example, E-cadherin binds with the greatest strength and specificity to E-cadherin, but it can also bind to N-cadherin [521] and certain integrins [361]. Hence the mixture of adhesion molecules on the two cells' surfaces (and the specificity and kinetics of the bonds between the molecules) will determine the strength of their adhesion. Adhesive differences between cell types can lead to self-sorting behavior based upon adhesion gradients, which contributes to epithelial cell organization in tissues [551]. Such cell sorting has been observed experimentally [41].

2.1.3 Subcellular structure

A cell is composed of a well-defined nucleus containing the cell's DNA, surrounded by cytosol (the liquid in the cell) and enveloped in a bilipid cell membrane. The cytoplasm contains organelles that carry out the cell's functions, such as the mitochondria (which synthesize adenosine triphosphate (ATP) from glucose and oxygen to provide energy to the cell) and endoplasmic reticulum (which provides ideal conditions for protein synthesis, folding, and transport), all supported by a *cytoskeleton* of microtubules and actin polymer fibers. See Figure 2.3. The bilipid membrane separates the cell from the microenvironment. It is permeable to the passive diffusion of small molecular species such as oxygen and glucose, actively pumps other molecular species (e.g., potassium and sodium) to maintain the cell's internal pH and chemical composition, and is impermeable to other, larger, molecules such as growth factors. Embedded in the membrane are a variety of macromolecules that pump smaller molecules (e.g., potassium) against gradients, exchange mechanical forces with the extracellular matrix, basement membrane, and other cells, and transmit microenvironmental information to the cell interior.

2.1.4 Cell cycle, proliferation, and apoptosis

Cell division is regulated by a highly regimented series of stages known as the *cell cycle*. In the first stage in the cell cycle, G1 (gap 1), the cell physically grows, proteins are