Multiscale Models of Breast Cancer Progression

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Abstract

Breast cancer initiation, invasion and metastasis span multiple length and time scales. Molecular events at short length scales lead to an initial tumorigenic population, which left unchecked by immune action, acts at increasingly longer length scales until eventually the cancer cells escape from the primary tumor site. This series of events is highly complex, involving multiple cell types interacting with (and shaping) the microenvironment. Multiscale mathematical models have emerged as a powerful tool to quantitatively integrate the convective-diffusion-reaction processes occurring on the systemic scale, with the molecular signaling processes occurring on the cellular and subcellular scales. In this study, we reviewed the current state of the art in cancer modeling across multiple length scales, with an emphasis on the integration of intracellular signal transduction models with pro-tumorigenic chemical and mechanical microenvironmental cues. First, we reviewed the underlying biomolecular origin of breast cancer, with a special emphasis on angiogenesis. Then, we summarized the development of tissue engineering platforms which could provide highfidelity *ex vivo* experimental models to identify and validate multiscale simulations. Lastly, we reviewed top-down and bottom-up multiscale strategies that integrate subcellular networks with the microenvironment. We present models of a variety of cancers, in addition to breast cancer specific models. Taken together, we expect as the sophistication of the simulations increase, that multiscale modeling and bottom-up agent-based models in particular will become an increasingly important platform technology for basic scientific discovery, as well as the identification and validation of potentially novel therapeutic targets.

Keywords

Multiscale modeling; Breast cancer; Hypoxia; Angiogenesis

INTRODUCTION

Breast cancer is one of the predominant cancers diagnosed among women, and the second leading cause of cancer death.[66] In the past, most experimental cancer research has focused on the genetic and molecular scale malfunctions which deregulate cell growth.[10] Understanding the deregulation of the wiring which controls central molecular programs is a daunting and multifaceted problem. These molecular pathways are large, and contain complex architectural features such as redundancy, feedback and crosstalk. [148] While this complexity ensures robustness and efficiency, it also complicates the reprogramming of

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signal flow and the interpretation of experimental findings. For example, Jones *et al.* showed in a study of pancreatic cancer patients that on average each patient had 63 genetic alterations spread throughout 12 core signaling pathways.[69] Thus, there was not a single dominant malfunction or pathway. Rather a combinatorial interplay of malfunctions acting in concert deregulated cellular function. This integration underscores the realization that cancer is a systems disease, even at the subcellular length scale.

Unfortunately, tumorigenesis involves far more than just malfunctions in signal transduction pathways in homogenous cell populations. Breast tumors are highly heterogenous, involving the simultaneous transmission and processing of many chemical and mechanical signals between multiple cell types within a time- and spatially-varying microenvironment. Furthermore, this cellular variety often includes diverse genetic populations within the same cell type. For example, Navin *et al.*[104] sequenced single cells in highgrade (III) ductal carcinomas and found a complex polygenomic population containing approximately 63% normal and 37% tumor cells, with a large fraction of leukocytes. Interestingly, within the same tumor, they identified four major genetically diverse tumorigenic cell subpopulations. Thus, understanding and ultimately reprogramming the integration of central programs such as proliferation, differentiation or death within multiple cell types, or genetic variants of the same cell type, in concert with the chemical and mechanical cues of the microenvironment is a grand challenge.

To attack a complex disease like breast cancer, we must build comprehensive experimental and computational tools which integrate intracellular signaling architectures with the extracellular microenvironment. Multiscale simulation methods, in combination with novel *in vitro* tissue engineering platforms, are rapidly evolving to meet this critical challenge. In this study, we review the current state of the art in cancer modeling across multiple length scales, with an emphasis on the integration of intracellular signal transduction models with pro-tumorigenic chemical and mechanical microenvironmental cues. First, we review the underlying biomolecular origins of breast cancer with a special emphasis on angiogenesis. Next, we summarize the development of tissue engineering platforms which could provide high-fidelity *ex vivo* experimental models to identify and validate multiscale simulations. Following that, we review top-down and bottom-up multiscale computational strategies that integrate subcellular networks with the microenvironment and tumorigenesis. We present models of a variety of cancers, in addition to breast cancer specific models. Thus, as our understanding of the complexity of these processes evolves, multiscale simulation could be a critical tool which provides fundamental biological understanding and potentially important clinical insight.

THE BIOMOLECULAR ORIGINS OF BREAST CANCER

Breast cancer is a highly heterogenous disease which can be broadly subdivided into three major subtypes: hormone receptor-positive tumors, ERBB2-amplified tumors and a third category collectively referred to as triple-negative tumors. The molecular understanding of each subtype, along with the possible treatments for each,[58] continues to evolve. High-throughput analytical technologies, such as gene expression profiling or rapid whole-genome sequencing, have been used to great effect to characterize the tumor type and microenvironment, [2,114] and specific gene signatures associated with stages of the disease.[25,104,115,147] The traditional tumor initiation hypothesis posits that genetic transforming events in single cells, e.g., TP53 mutations[110] or epigenetic changes,[64] leads to clonal expansion and the accumulation of additional genetic changes. However, mutations in genes classically associated with breast cancer, e.g., BRCA1, BRCA2 and TP53, account for less than 25% of the excess risk associated with family history.[115] Thus, there are likely other transformation pathways that initiate the disease. For example, this traditional view

has recently been challenged by the cancer stem cell (CSC) hypothesis in which differentiated cancer cells, which are unable to self-renew, are the progeny of a population of self-renewing CSCs.[16,106,115]. These tumorigenic cells can then recruit (or phenotypically transform) many other cell types which collectively form the microenvironment of the growing tumor (Fig. 1). Interactions between the tumorigenic cells and the microenvironment, and even the cellular composition of the microenvironment, is a complex function of many factors.[73] It is thought that autocrine and bidirectional paracrine signaling regulates the tumorigenic cell population (including CSCs), and these cells in turn secrete factors which influence the makeup and behavior of the microenvironment.[82,116] However, soluble signals are likely not the only important cues. Physical changes, solid stresses, matrix stiffness, fluid pressure and other biomechanical forces have also been implicated in tumorigenesis and may influence the recruitment of other cell types including circulating tumor cells (CTCs), fibroblasts and immune cells (recently reviewed by Shieh et al.[127] and Lu et al.[86]). The complexity of the tumor microenvironment may even play a critical role in drug resistance (see Correia et al.[29]). However, CSCs/CTCs and their respective role in driving tumorigenesis remains controversial. Yet another hypothesis, which builds upon an older idea, is that tumorigenesis is actually a malfunctioning wound-healing process.[96] Whatever the initiation events and source of heterogeneity, it is agreed that the complexity of breast tumorigenesis complicates our understanding of the disease, and ultimately limits the development of effective targeted treatment options.

The transition from localized ductal carcinoma to invasive and ultimately metastatic breast cancer is a critical milestone impacting the clinical management and outcome of the disease. One of the central programs associated with this transition is angiogenesis (Fig. 1). Tumor angiogenesis is stimulated by many factors, including reduced oxygen tension (i.e., hypoxia) which up-regulates the secretion of proangiogenic signaling molecules by tumorigenic cells.[56] Vascular endothelial growth factor (VEGF) is the most potent angiogenic factor secreted by tumor cells in response to hypoxia.[39,43] Anti-angiogenic therapies aimed at disrupting the molecular coupling between hypoxia, VEGF signaling, and tumor angiogenesis initially showed great promise.[40] Several of the regulatory axes which control VEGF expression in the microenvironment, for example the role of oxygen tension, are relatively well understood. Oxygen in the microenvironment is sensed by hypoxia inducible factor 1α (HIF1α) and the generation of reactive oxygen species (ROS).[79,100] HIF1α mediates the initial phase of the angiogenic program by forming a transcriptionally active complex with HIF1β and co-activators such as p300. The stability of the HIF1α subunit is oxygendependent. [117] In normoxic conditions, hydroxylation at two prolyl residues (P402 and P564) by PHD proteins promotes the association of HIF1α with the Von Hippel- Lindau (VHL) E3 ubiquitin ligase and subsequently leads to degradation. An additional hydroxylation site at N803 near the C-terminus of HIF1α is regulated by the asparaginyl hydroxylase FIH. Hydroxylation at N803 does not influence stability; rather, it blocks the interaction of the HIF1α C-terminal domain with transcriptional co-activators such as p300. Activated HIF1 up-regulates the expression of many factors including VEGF and Interleukin-8 (IL-8).[146] On the other hand, ROSpromotes nuclear factor κB (NF-κB) activation.[100] NF-κB also regulates both VEGF and IL-8 expression. [100,146] The exact relationship between ROS and NF-κB activation is unclear; ROS has been hypothesized to activate serine kinases which in-turn phopshorylate the N-terminal serine residues (S32/S36) on IKK.[44] Unfortunately, the initial success of the anti- VEGF-A monoclonal antibody bevacizumab has been reexamined in light of clinical evidence suggesting anti- VEGF therapy often prolongs patient survival by only months, without offering an enduring cure.[72] Studies have also emerged questioning the overall survival advantage of bevacizumab in combination with chemotherapeutics, [98] while other studies suggested that even short-term exposure to potent anti-angiogenic therapies might actually induce invasiveness.[36,108] These studies, in combination with potential safety concerns,[27] led the US Food and Drug

Administration (USFDA) to remove the breast cancer indication from the bevacizumab label.

The finding that anti-VEGF therapy may induce invasiveness seemingly contradicts years of dogma suggesting VEGF-induced vessel recruitment is essential for cancer progression. Carmeliet and coworkers recently reviewed two of the leading hypotheses explaining this apparent contradiction.[84] The first has suggested that reduced angiogenesis selects for a hypoxia-tolerant tumorigenic population that is better adapted to the low-oxygen microenvironment.[18] These hyper-tolerant tumorigenic cells thrive in the noxious microenvironment by adapting their metabolism or escape by inducing invasive programs such the epithelial mesenchymal transition (EMT).[18] The role of EMT in cancer progression and metastasis has long been recognized.[55,140] However, this population of hyper-tolerant tumorigenic cells may also recruit other vascular precursor cell types, for example angiocompetent bone marrow-derived cells,[52] or co-opt existing vasculature that is not inhibited by anti-VEGF therapy. [15] Ebos and coworkers suggested a second hypothesis where VEGF inhibitors induce a chronically inflamed state characterized by the expression of several factors including stromal cell-derived factors 1-alpha (SDF1a), placenta growth factor (PlGF), interleukin-6 (IL-6), erythropoietin, osteopontin, and other cytokines.[35] These cytokines may then recruit angiogenic bone marrow-derived endothelial and myeloid progenitors,[70] many of which express vascular endothelial growth factor receptor 1 (VEGFR1), thus their recruitment is not blocked by VEGF inhibitors.[70] Both of these hypotheses involve the recruitment of immunomodulatory cell types by the secretion of cytokines and other factors. The integration between the immune system, inflammation and cancer progression (including the modulation of the CSC population) is an emerging area with classical roots.[34] The immune system can both inhibit and stimulate tumorigenesis, where these influences are mediated by complex mechanisms.[93] Inflammatory signals, such as Interleukin-6 (IL-6) and IL-8, are secreted by many cell types in the microenvironment.[147] IL-6 is known to promote breast cancer progression,[125,126] and serum levels of both IL-6 and IL-8 correlate with patient outcome.[14,124] Interestingly, both IL-6 (*via* the GP130 receptor) and IL-8 (*via* the CXCR1 receptor) have also been shown to directly regulator breast cancer stem cell (BCSC) self-renewal.[64] The expression of both of these cytokines is regulated by NF-κB,[12] thereby potentially linking this critical signaling axis with ROS formation in hypoxic environments.

Ex Vivo Experimental Models

The development of effective anti-angiogenic therapies depends critically upon a comprehensive understanding of proliferation and vascularization programs and the interaction of these programs with the microenvironment. Multiscale simulation tools in combination with high-fidelity *ex-vivo* experimental models can help unravel this complexity.[111] However, multiscale models require fine-grained training and validation data to be successful. Unfortunately, wideranging but fine-tuned experimental control of the receptor signaling cascades involved in angiogenesis or other tumorigenic processes is not possible with current *in vitro* and *in vivo* approaches. For example, conventional angiogenesis models (e.g., tube formation on Matrigel) fail to capture: (i) the intrinsic, threedimensional morphology and diffusion-limited formation of intratumor niches, (ii) microscale integration of multiple cell types within physiologically relevant architectures, and (iii) coupling to a vascular interface that provides systemic convective transfer of endocrine signals and other cellular nutrients. Tissue engineering approaches to model tumor physiology have recapitulated the reaction-diffusion processes of solid tumors and begun to elucidate the microphysiological details of the angiogenic and other tumorigenic processes. These advancements have been enabled by new synthetic materials,[88] development of microfluidic lab on a chip technologies[33,63] as well as a new appreciation

for the significant role played by the microenvironment in shaping tumor progression.[86] The integration of microfluidic and three dimensional tissue engineering technologies permits control over and monitoring of the soluble microenvironment experienced by cells.[21–26] Nelson *et al.*[105] developed one of the first three-dimensional patterning techniques to construct multicellular epithelial tissues in three-dimensional gels composed of extracellular matrix (ECM) proteins. Using this patterning technology, they later explored the signaling forces driving cell organization in engineered three dimensional mammary ducts[101] as well as how complex interactions between mammary progenitor cells and the microenvironment drive cell fate decisions.[75] Using three-dimensional polymeric scaffolds to mimic the tumor ECM, we recently showed that dimensionality (i.e., two-dimensional vs. threedimensional), hypoxia, and integrin engagement play a critical role in VEGF and IL-8 up-regulation.[42,43] Zheng *et al.*[153] created *ex-vivo* microvascular networks using human umbilical vein endothelial cells (HUVECs) seeded into microfluidic circuits formed *via* soft lithography in a type I collagen gel. They quantified sprout formation following exogenous administration of vasculogenic medium throughout the device. Seok and coworkers used a similar three-dimensional microfluidic strategy to explore sprouting in the presence of angiopoietin 1 (ANG-1) and VEGF gradients. [68,128] Engineered culture systems could advance studies of tumor vascularization by faithfully replicating the *in vivo* microenvironment, while providing highly quantifiable, and controlled conditions. Microfluidic devices have also been used to reconstruct realistic microenvironmental mimics to study other processes important in breast tumorigenesis e.g., differentiation and migration.[62] These experimental tools and others, such as bead-based methods,[103] when combined with mathematical models of signaling driving the evolution of the microenvironment, could unravel the complexity of tumor vascularization and perhaps identify molecular targets for improved proangiogenic therapies.

MULTISCALE MODELING METHODS IN CANCER

Many factors act in concert to drive tumor formation. These forces act across multiple length and time scales, involve heterogenous cell populations and involve both biophysical and biochemical cues. To understand how these disparate forces drive tumor formation generally, and breast cancer tumorigenesis in particular, we need to develop predictive multiscale models. Multiscale models of tumorigenic processes e.g., growth-factor induced proliferation or angiogenesis dynamics are not new. Mathematical models exploring this space of problems have been developed since the 1970s (see Quatub *et al.*[119] for a review angiogenesis models). A wonderful compilation of recent work in multiscale modeling has been organized in a book edited by Deisboeck and Stamatakos.[31] The individual chapters (authored by several groups) describe the application of agent based and continuum modeling strategies to study several cancer types, including breast cancer. Moreover, several journals have dedicated special issues to multiscale simulation methods and their application to cancer modeling.[121] While the objectives of multiscale simulation studies have not changed in several decades, current models are significantly more sophisticated. This increased sophistication has largely been driven by increased biological understanding and the rapid increase in computing power.

Multiscale strategies can broadly be organized into continuous, discrete and hybrid approaches. Continuous approaches use continuum mechanical principles encoded in partial differential equations (PDEs) or integral partial differential equations (IPDEs) to describe the variation of population-averaged phenomena, e.g., tumor cell density as a function of space and time. Continuum models offer the advantage of easily describing whole tumor dynamics, including complex physical phenomena such as interstitial pressure gradients and convective transport from the tumor.[65] For example, Murray and coworkers used continuum approaches to model prostate cancer[137] and many aspects of glioma formation,[136] including

the response to treatment.[135] More recently, Swanson and colleagues used continuum approaches to model glial progenitor cell recruitment.[94] Continuous approaches have also been used to explore therapeutic antibody distribution in tumors,[142] as well as the design of therapeutic antibodies.[120] While these and other continuum studies have generated nontrivial insights, continuum models are limited to a population-averaged picture of the tumor. This is an issue if you are interested in population distributed behavior at the cellular and subcellular length scales, or the behavior of your system is strongly stochastic. On the other hand, discrete approaches such fully stochastic simulations, can predict emergent properties generated by interactions between individual cells.[23] Fully stochastic methods, such as the next subvolume method (NSM), naturally integrate stochastic reaction dynamics with physical models.[57] Unfortunately, stochastic methods such as NSM typically scale poorly with problem size.

Agent Based Models (ABMs)

In between continuum and fully discrete approaches are hybrid strategies. Perhaps the best known hybrid strategy in the cancer and complex systems community is agent based modeling (ABM).[17] ABMs are a class of simulation in which combinations of autonomous actors or *agents* are embedded in a spatially and temporally varying computational universe. Both the agents and universe may have *state*, meaning variables or variable combinations which describe the current configuration of the system. The stateful agents individually interact with the universe (and each other) using predefined rules. These interactions can be twoway, i.e., the state of the agents can be informed by the universe (often governed by continuum mechanics), and conversely the state of the universe can inform the agents (Fig. 2). Integration between the behavior of the agents and the microenvironment occurs naturally by making the behavior rules functions of spatially or temporally distributed microenvironmental variables. Arguably, ABMs have had the largest impact simulating morphogen-induced developmental programs [50,141] as well as immunological processes such as cell trafficking.[9] However, ABMs have also proven useful in modeling tumorigenesis,[1,134] subprocesses such as normal and pathological angiogenesis[30,81] and microvascular patterning.[112] ABMs have also been used extensively in ecology,[51] epidemiology,[22] crowd behavior[102] as well as non-biological fields such as transportation management.[17] Thus, ABM is a powerful technique with wide applicability to a broad spectrum of problems, not just modeling cancer progression.

There are two schools of thought governing ABM rule formulation. Top-down approaches, which have traditionally been the most popular strategy, encode system attributes as coarse-grained empirical rules which describe global control mechanisms. Often these rules are based on experimental observations, thus topdown ABMs can predict sophisticated cellular behavior without mechanistic information.[23] Furthermore, software packages, e.g., NetLogo[129] or CompuCell[4] facilitate ABM formulation and simulation, making this strategy easy to implement. On the other hand, bottom-up approaches use mechanistic signal transduction pathway models to inform the behavior of agents. Each agent in the simulation is equipped with these signaling networks. Thus, the signaling profile of each agent can vary as a function of time and position, within the microenvironment. This integration allows agents to make complex decisions which vary as the extracellular microenvironment varies. Moreover, if these signaling programs result in secretion, the agents can transform the local extracellular matrix or initiate autocrine or paracrine signaling programs. The advantage of a bottom-up strategy is the direct coupling of agent behavior with cellular or subcellular signaling programs. Arguably one of the most advanced examples of a bottom-up biophysical tumor simulation is the recent vascularized tumor growth model of Perfahl *et al*.[113]. In the Perfahl *et al*. study, a comprehensive simulation that integrated several biophysical and biochemical facets of tumorigenesis e.g., blood flow, angiogenesis, vascular

remodeling, extracellular transport and nutrient-dependent cell cycle dynamics was used to explore three-dimensional tumor formation. While the solid tumors simulated were not breast tumors, the strategy used in the Perfahl *et al*. study could be easily adopted to model breast tumorigenesis. Of course the Perfahl *et al*. study was build upon or extended several other important previous multiscale studies.[91,107] The integration of subcellular networks with macroscopic tumor formation, also potentially allows bottom-up ABMs to be useful as *in silico* surrogates for therapeutic target identification, or ultimately to understand the morphological outcome of cellular mutations. For example, Rejniak *et al*. used the IBCell framework to simulate the formation of epithelial structures e.g., hollow acinar structures which were qualitatively consistent with three dimensional MCF10A cell culture studies.[122] This study was important in two ways. First, it an excellent case study of the integration of experimental tools with multiscale simulation. Second, the authors performed a parameter sampling calculation that identified regions of distinct epithelial morphologies. These possible configurations were then validated with engineered MCF10A cell lines. The latter aspect of this study firmly established that bottom- up multiscale models could be used as predictive tools. However, including mechanistic information can also be a disadvantage; intracellular signal transduction models are difficult to formulate, identify and validate. This is especially true in breast cancer because of the multiple cytokine and growth-factor signaling axes involved in the disease.[76] Thus, one of the central challenges to using bottom-up ABMs is the identification of the signal transduction models used in the rule sets. Typically, these models are formulated as a coupled system of nonlinear ordinary differential equations (ODEs), however many other model formulations could be possible.[71]

To formulate and solve ODE signaling models requires a deep understanding of both network structure and model parameters. The rates of biochemical or biophysical transformations within ODE models can be described using a variety of kinetic formulations, e.g., mass-action kinetics.[24] These various kinetic forms have a variety of parameter types that must be estimated or measured. The parameter estimation problem is often very difficult, given the underdetermined and noisy nature of most training data sets. Moreover, signal transduction models typically exhibit complex behavior with respect to inputs and their parameters. For example, models of growth factor, hormone signaling, differentiation and MAPK signaling all showed threshold or switch-like behavior.[7–138]. Thus, it is often impossible to uniquely identify parameters in signaling models, even with extensive training data.[46] Despite identification standards[47] and the integration of model identification with experimental design,[11] parameter estimation remains challenging. Towards this issue, a number of groups have turned to ensemble methods. Instead of identifying a single (but uncertain) model, the goal of an ensemble approach is to identify a family of models consistent with, and constrained by, the available experimental data. This strategy has been used in systems biology and other fields like weather prediction to identify parameter rich models using incomplete or sparse data.[74–109] Their central value is the ability to constrain model predictions despite uncertainty in the model parameters (and sometimes structure). For example, Sethna and coworkers showed that an ensemble of growth factor signaling models gave good predictions despite incomplete parameter information (sometimes only order of magnitude estimates).[20] They further showed that model ensembles were predictive using many different mathematical model formulations.[54] Model ensembles have also been hypothesized as a general coarse-grained means to capture population distributed phenomena when stochastic simulation is too expensive. For example, the population specific translation regulation[77] or the response of a patient population to treatment.[87] There are several numerical techniques to generate model ensembles. Battogtokh *et al*. introduced a Metropolis-type random walk strategy[97] to estimate an ensemble of models describing the quinic acid gene cluster of *Neurospora crassa*.[13] This Monte Carlo strategy was later modified by Tasseff *et al*. to control for ensemble correlation in models of prostate cancer,[138] and later Retinoic Acid (RA) induced

differentiation of hematopoietic precursors cells.[139] A similar Markov-Chain Monte Carlo technique was developed by Song *et al.* to generate a family of models describing Neutrophil trafficking in Sepsis.[132] Other strategies such as the Pareto optimal ensemble technique (POET), a multiobjective optimization strategy which uses simulated annealing to sample parameter space, have also been proposed. [130,131] POETs has been used, in combination with cross-validation, to generate predictive ensembles for several networks including fundamental programs such as translation initiation.[77] Taken together, identification of intracellular signal transduction models that are parameter rich will continue to be a challenge. However, ensembles are one strategy to develop reasonably predictive models which could be useful for ABM simulations, despite uncertainty.

Perhaps the more fundamental challenge to developing predictive signal transduction models is representing the signaling network architecture. Yeast Two- Hybrid (Y2H),[41], Fluorescence Resonance Energy Transfer (FRET)[150] or Chromatin Immunoprecipitation (ChIP) combined with DNA-microarrays (ChIP-chip) or high-throughput DNA-sequencing (ChIP-seq) techniques[92] have all been used to estimate protein–protein or protein–DNA interactions. These techniques when combined with low-throughput immunoprecipitation have been the basis for most experimental network discovery. Computational motif discovery,[99] network discovery and reconstruction using high-throughput data sources[145,149] or text mining, [3,38] have also contributed significantly to network identification. These studies and many others have led to comprehensive on-line network databases such as STRING,[67] NetworKIN,[80] PhosphoSitePlus[60] or KEGG[6] which continue to evolve as new information is made available. On-line model repositories such as the BioModels database[78] have also been created to archive published signal transduction models. Thus, as more network architecture information becomes available, and model development continues to evolve perhaps the challenge of developing comprehensive signal transduction simulation models will decrease. However, for the foreseeable future, biologically realistic network models are likely to be parameter rich and data poor, even with the advent of advanced analytical techniques.

Despite identification challenges, there are several examples where bottom-up strategies have been used to integrate subcellular data with the microenvironment. [32] Deisboeck and coworkers developed a number of ABM simulations exploring growth-factor signaling within brain and non-small cell lung cancer (NSCLC) tumors. An ODE-based epidermal growth factor (EGF) signaling model was embedded within two- and three-dimensional computational domains, where the spatial-temporal dynamics of the microenvironment domain was governed by PDEs. This framework was then used to explore a number of complex questions: the role of epidermal growth factor receptor (EGFR) density in tumor progression,[8] the influence of genetic instability in tumor heterogeneity,[152] the components that control the proliferation-to-migration switch for brain tumors,[151] and the role of EGF and TGFβ signal integration in non-small cell lung cancer.[144] Macklin *et al.* used an ABM approach to investigate breast ductal carcinoma *in situ* (DCIS),[90] using patient-specific molecular and cellular measurements to calibrate their model. Likewise, Frieboes *et al.* used multiscale modeling to identify specific functional relationships linking tumor growth and regression to the underlying phenotype of breast cancer following chemotherapy.[45] Simulations of the factors controlling tumor shape and morphology is another area where ABMs have made an impact. For example, Engler *et al.* used multiscale modeling to investigate how emergent properties of adhesion-directed multicellular structures sculpt the tissue, promote its functionality, and maintain its homeostasis.[37] While the majority of bottom-up decision networks are mechanistic, non-mechanistic network models have also been used to guide agent behavior. For example, Gerlee and Anderson used a neural network formulation where extracellular variables formed the input layer, intracellular variables were the hidden layer and phenotype was the output layer.[48] The neural-network

agent framework was used in several studies of the general properties of invasion and tumorogenesis,[5,49] including the response of the tumor to treatment.[59] Potentially, other hybrid mechanistic models, such as discrete logic models, could also be used in the ABM rules.[123]

There is growing enthusiasm for using signaling assisted multiscale models as tools for therapeutic target discovery and validation.[143] However, the signaling models used to date have not been comprehensive, typically containing perhaps two abstracted pathway architectures at most. Thus, while the concept of using bottom-up ABMs for drug discovery is intriguing, the description of the biology must be significantly expanded to capture the intricate responses of signaling architectures to perturbation. Increasing the level of detail of the signaling architectures used by agents brings several challenges. We have already mentioned the challenges of network model identification. Assuming we already have identified network models, the next big challenge is then the scaling performance of the simulation. While the exact performance of bottom-up ABM simulations is problemspecific, in the worst case we would expect exponential scaling with the number of agents. Thus, detailed simulations using many bottom-up agents, each equipped with multiple decision networks, is not tractable on single processor machines. However, this is a common issue faced in many multiscale modeling application domains. For example, combustion applications often have chemical reaction networks with hundreds or thousands of species which are coupled to turbulent flow models. For these problems, Pope and coworkers developed the *in situ* adaptive tabulation (ISAT) algorithm which minimizes expensive function updates. Interestingly, the ISAT strategy has resulted in speed-ups of up to a thousand-fold for complex combustion calculations.[85] While ISAT has not been applied to bottom-up ABMs, this and other highperformance computing strategies could be adapted to facilitate increasingly detailed multiscale simulations.

CONCLUSIONS

Breast cancer initiation, invasion and metastasis span multiple length and time scales. Molecular events at short length scales lead to an initial tumorigenic population, which left unchecked by immune action, acts at increasingly longer length scales until eventually these cells escape from the primary tumor site. This series of events is highly complex, involving multiple cell types interacting with (and shaping) the microenvironment. Multiscale mathematical models have emerged as a powerful tool to quantitatively integrate the convective-diffusion-reaction processes occurring on the systemic scale, with the molecular signaling processes occurring on the cellular and subcellular scales. In this study, we reviewed the current state of the art in cancer modeling across multiple length scales, with an emphasis on the integration of intracellular signal transduction models with pro-tumorigenic chemical and mechanical microenvironmental cues. First, we reviewed the underlying biomolecular origin of breast cancer, with a special emphasis on angiogenesis. Then we summarized the development of tissue engineering platforms which could provide highfidelity *ex vivo* experimental models to identify and validate multiscale simulations. Lastly, we reviewed top-down and bottom-up multiscale strategies that integrate subcellular networks with the microenvironment. Taken together, we expect as the sophistication of the simulations increase, that multiscale modeling and bottom-up agent-based models in particular will become an increasingly important platform technology for basic scientific discovery.

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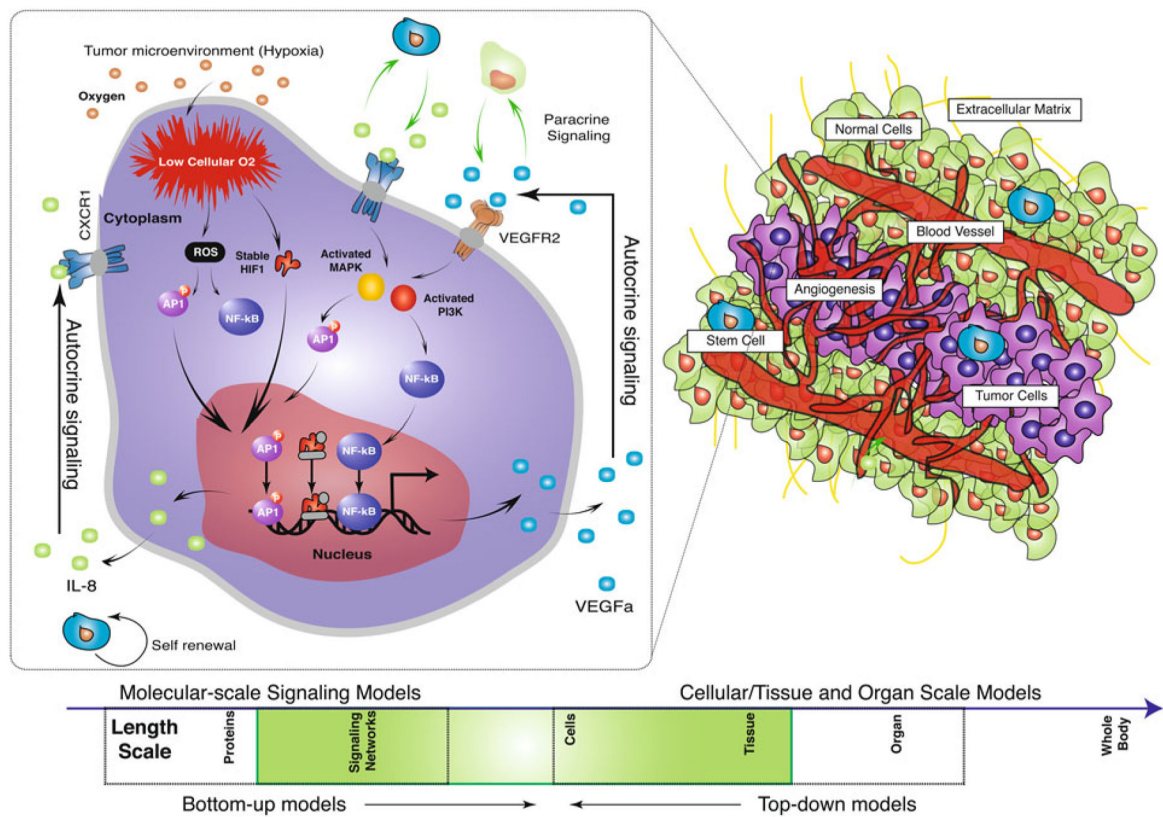
FIGURE 1.
Schematic of the tumor microenvironment. Breast cancer initiation, invasion and metastasis span multiple length and time scales. Molecular events at short length scales lead to an initial tumorigenic population, which left unchecked by immune action, acts at increasingly longer length scales until eventually the cancer cells escape from the primary tumor site. One of the central programs associated with this transition is angiogenesis. Tumor angiogenesis is stimulated by reduced oxygen tension (i.e., hypoxia) which up-regulates the secretion of pro-angiogenic signaling molecules, e.g., VEGF, Interleukin-6 (IL-6) and Interleukin- 8 (IL-8) by tumorigenic cells and other cell types in the tumor microenvironment. These signals then initiate autocrine and paracrine programs which shape the chemical, mechanical and cellular composition of the microenvironment.
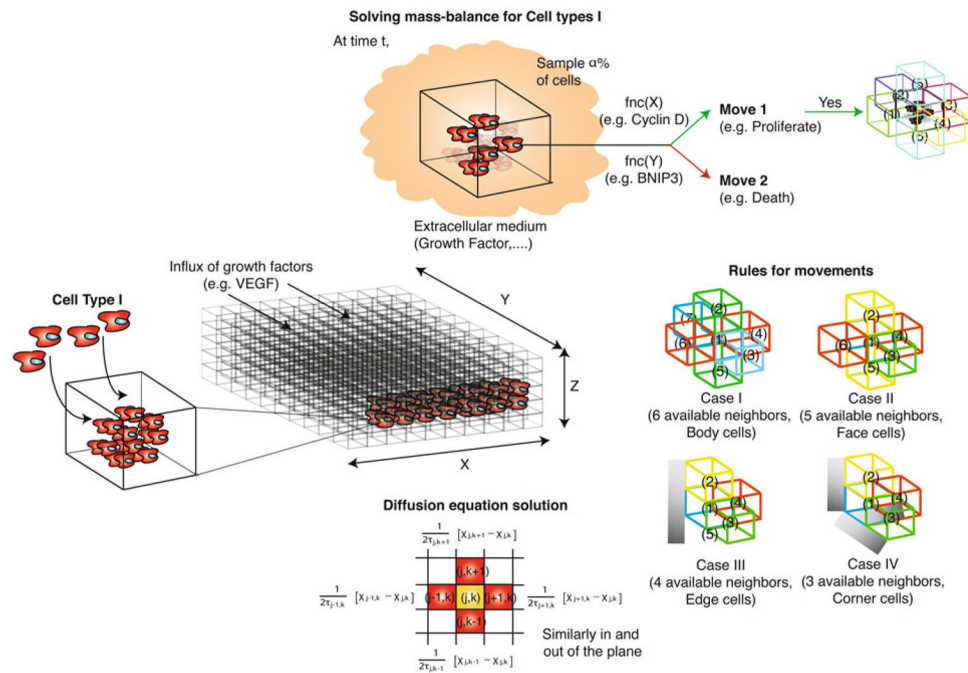
FIGURE 2.
Schematic of a generic bottom-up ABM strategy. A three-dimensional computational domain representing the microenvironment is discretized into well-mixed microcompartments. The extracellular state e.g., the concentration of pO_2 or VEGF in each of the microcompartments is governed by the solution of continuum mass balances equations (partial differential equations). Agents representing different cell-types, each equipped with perhaps many signal processing networks, are embedded into the computational microenvironment and allowed to evolve according to rules that are functions of the output of the signaling networks. The agents make decisions about possible actions e.g., move, proliferate, differentiate *etc.* by evaluating the network models. Thus, the decisions of the agents depend upon both the position and temporal state of the agent.