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REVIEW

Modeling head and neck cancer stem cell-mediated tumorigenesis

Alexander T. Pearson[1] · Trachette L. Jackson[2] · Jacques E. Nör[3,4,5,6]

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Abstract A large body of literature has emerged supporting the importance of cancer stem cells (CSCs) in the pathogenesis of head and neck cancers. CSCs are a subpopulation of cells within a tumor that share the properties of self-renewal and multipotency with stem cells from normal tissue. Their functional relevance to the pathobiology of cancer arises from the unique properties of tumorigenicity, chemotherapy resistance, and their ability to metastasize and invade distant tissues. Several molecular profiles have been used to discriminate a stem cell from a non-stem cell. CSCs can be grown for study and further enriched using a number of in vitro techniques. An evolving option for translational research is the use of mathematical and computational models to describe the role of CSCs in complex tumor environments. This review is focused discussing the evidence emerging from modeling approaches that have clarified the impact of CSCs to the biology of cancer.

Keywords Head and neck squamous cell carcinoma · Mathematical modeling · Mouse models of human cancer · Cancer stem cells · Cell culture · Orospheres · Statistical models

Abbreviations

HNSCC	Head and neck squamous cell carcinoma
HPV	Human papilloma virus
CSC	Cancer stem cell
PDX	Patient-derived xenograft

Introduction

Head and neck cancers are a heterogeneous group of cancers arising in the epithelial tissue from the paranasal sinus, lip, oral cavity, nasal cavity, pharynx, and larynx. In 2014, an estimated 55,070 new cases of oral cavity, pharyngeal, and laryngeal cancers occurred in the USA [1] and 400,000–600,000 annual cases worldwide [2]. Head and neck squamous cell carcinoma (HNSCC) is the most common histologic subtype, comprising approximately 90 % of the tumors of the head and neck region [3]. Other histologic subtypes including melanoma, adenocarcinoma, and mucoepidermoid, acinic, and adenoid cystic carcinoma also occur, albeit with much lower frequencies [4, 5].

The most common historical risk factors for HNSCC are alcohol consumption and tobacco use, which contribute to approximately 75 % of cancers [6–8]. High risk strains of human papilloma virus (HPV 16, 18) have recently

✉ Alexander T. Pearson
pearsona@med.umich.edu

✉ Jacques E. Nör
jenor@med.umich.edu

1 Division of Hematology/Oncology, Department of Internal Medicine, University of Michigan School of Medicine, 1500 E. Medical Center Dr., SPC 5848, Ann Arbor, MI 48109-5848, USA

2 Department of Mathematics, University of Michigan School of Literature, Sciences, and the Arts, Ann Arbor, MI, USA

3 Department of Restorative Sciences, University of Michigan School of Dentistry, 1011 N. University Rm. 2309, Ann Arbor, MI 48109-1078, USA

4 Department of Otolaryngology, University of Michigan School of Medicine, Ann Arbor, MI, USA

5 Department of Biomedical Engineering, University of Michigan College of Engineering, Ann Arbor, MI, USA

6 Comprehensive Cancer Center, University of Michigan, Ann Arbor, MI, USA

presented as an emerging risk factor [9]. HPV-associated HNSCC has a favorable clinical profile compared to tobacco- and alcohol-associated HNSCC [10].

Treatment decisions for HNSCC are complex, and a multidisciplinary approach is recommended according to US guidelines [3]. Treatment recommendations are based on cancer stage [11], location, and histological features. Treatment may include surgical resection, radiation therapy, chemotherapy, or a combination of these modalities. Treatment is complicated by a high rate of therapy-related morbidities [12], including swallowing changes, nutritional complications, and airway compromise. Medical oncology innovation has been slow, with only one new agent (cetuximab) being approved for HNSCC in the last 15 years [13, 14], and consequently survival rates for patients with head and neck cancers have improved less than those for patients with other malignancies [15]. Head and neck cancer is responsible for approximately 350,000 global deaths from cancer annually [1]. Much of this HNSCC mortality is due to cancer recurrence, with 20–40 % of patients developing loco-regional recurrence and 5–20 % developing distant metastases at 2 years [16].

Molecular pathogenesis of HNSCC

There are numerous molecular pathways contributing to the pathogenesis of HNSCC [17]. Generally, carcinoma cells arise from premalignant precursor lesions following the activation of proto-oncogenes or inactivation of cancer suppressors, respectively [18]. A majority of HNSCC cases have loss of heterozygosity at chromosome regions 9q21 or 3p14 [19]. Telomerase is reactivated both in precursor lesions and in HNSCC [20], thereby assisting in the preservation of genetic changes. Epithelial growth factor receptor (EGFR) expression is seen in the preponderance of HNSCC [21], and overexpression of EGFR portends a poor clinical outcome [22]. Interleukin-6 (IL-6) has also been shown to have a strong correlation with clinical outcomes [23]. Endothelial cells secrete IL-6 in response to inflammatory stimuli [24], and IL-6 activates its downstream target signal transducer and activator of transcription 3 (STAT3), which is activated in head and neck cancer [25]. The pro-angiogenic chemokine C-X-C motif Ligand 8 (CXCL8 or IL-8) has also been shown to increase endothelial cell proliferation and migration [26, 27], and is produced by HNSCC cells [28].

A recent analysis by the Cancer Genome Atlas provided a genomic landscape for HNSCC [29]. They described distinct profiles for HPV- and smoking-related HNSCC. In this study, HPV-related tumors exhibited mutations in oncogene PIK3CA, loss of TRAF3, and amplification of the cell cycle gene E2F1, while smoking-related tumors exhibited loss-of-function TP53 mutations and CDKN2A inactivation, as well as copy number alterations. Cigarette smoking produces reactive oxygen species [30], which damage the cellular membranes, inducing DNA damage and activating oxidative-sensing cellular pathways [31, 32]. These activated signaling pathways lead to inflammatory gene activation, including CXCL8 (interleukin-8), mitogen-activated protein kinase (MAP kinase), nuclear factor-κB (NK-κB), signal transducer and activator of transcription (STAT)-3, and tumor necrosis factor (TNF)-α [33–38]. Smoking damage induces field cancerization throughout the aerodigestive tract and increases the risk for subsequent second primary cancer formation [39]. Human papillomaviruses in infected head and neck tissue express viral oncoproteins E6 and E7, which ubiquitinate tumor suppressor proteins p53 and retinoblastoma (pRb), respectively [40–44].

Stem cells in head and neck cancer

Pluripotent stem cells have been extensively described as an essential component of normal human tissue [45, 46]. The fundamental feature of a stem cell is its ability to recapitulate a heterogeneous organ from a single progenitor cell. This function can be activated in response to growth stimuli, injury repair, or organogenesis. The cancer stem cell hypothesis extends this principle to describe key phenomena observed during tumor growth. Cancer stem cells (CSCs) are defined as having properties of tumorigenesis, self-renewal, and the capacity to differentiate [47, 48]. When a CSC divides, it may either undergo self-renewal (creating two self-same daughter cells), or asymmetric division (creating one CSC and one more differentiated cell). Factors that shift the balance between self-renewal and asymmetric division may change the ultimate proportion of CSCs within the full tumor population (Fig. 1).

For the purposes of the study, we must also have the ability to identify, enrich, or isolate this population by some means. Cancer stem cell populations have now been identified in a variety of tumors [49–51], including head and neck squamous cell carcinoma [52]. Whether head and neck CSCs arise from changes within stem cells or from non-stem cells gaining stem cell properties remains an open topic of debate [53, 54].

Stemness markers in HNSCC

Head and neck CSCs are an important research focus because of their unique pathogenic properties. Other terms including “cancer progenitor cells”, “tumorigenic cells”, and “tumor initiating cells” are used to describe CSCs. However, “cancer stem cells” generally remains the

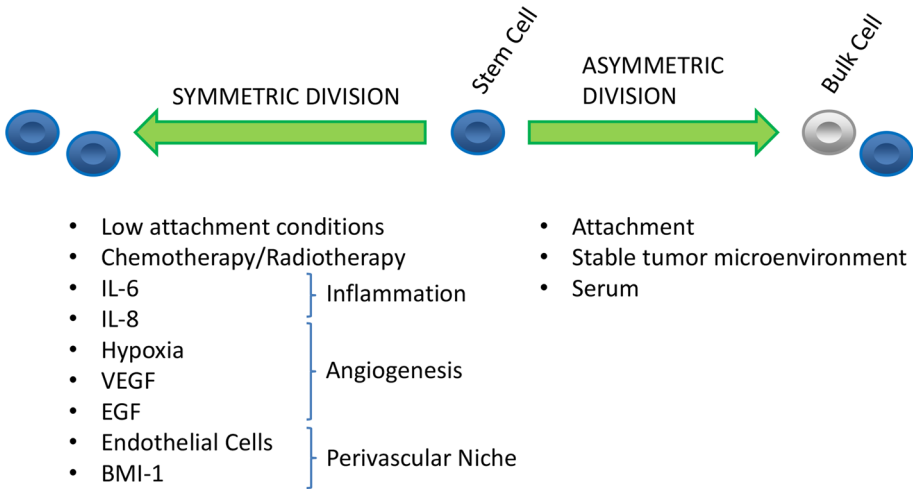

Fig. 1 Factors affecting the self-renewal or differentiation of head and neck CSCs

preferred term and describes shared functional relationships of CSCs with embryological stem cells [55]. The three primary embryological stem cell regulators of undifferentiated state and symmetrical cell division are Oct-4 [56], Nanog [57], and Sox-2 [58, 59]. Octamer-binding transcription factor-4 (Oct-4) is a POU-domain family transcription factor involved in maintaining developmental potency in embryonic stem cells [60]. Similarly, Nanog is a homeodomain transcription factor responsible for blocking cell differentiation and maintaining pluripotentiality of embryonic stem cells [57]. Sex determining region Y-box 2 (Sox-2) is a member of the SOX protein family, which shares conserved DNA-binding domains with the high mobility group family of chromosomal proteins. Sox-2 aids in cell fate regulation in early development [58] and has also been proposed as a squamous cell cancer histology-specific lineage marker [61].

Head and neck cancer cells enriched for CSCs using sphere assays had increased expression of Nanog, Oct-4, and Sox2 [62]. Furthermore, positive correlations of Nanog and Oct-4 with tumor stage at presentation and poor clinical prognosis have been described [63]. Nanog and Oct-4 may also play a role in chemotherapy resistance, as their expression levels are both increased in cisplatin-resistant cell lines [64]. The "pro-stemness" gene B cell-specific Moloney murine leukemia virus-insertion site (BMI)-1 is expressed in populations of HNSCC CSCs and is largely absent in non-CSC populations [65]. Hypoxic conditions have been shown to enhance stem cell properties of tumors via hypoxia inducible factors (HIF) and increased expression of Oct-4, as well as other pathways [66, 67].

CSC therapeutic resistance

Head and neck CSCs exhibit enhanced tumorigenicity and exhibit a higher yield with fewer cancer cells compared to non-CSCs [52, 65, 68]. Even though they represent less than 10 % of the total tumor population, CSCs can initiate full tumors with as few as 50 cells [69]. Similarly, isolated non-stem cells are much less likely to form tumors [70]. In addition to their tumorigenic properties, head and neck CSCs are resistant to therapeutic intervention with traditional cytotoxic agents. CSCs are differentially more resistant to both the chemotherapy and radiation therapy that would typically target proliferative cells [71]. Cisplatin and fluorouracil chemotherapies have both been shown to increase the population of head and neck CSCs, implying at least a differential effect of chemotherapy [72, 73]. Cisplatin also upregulates the stem cell marker BMI-1 [74] as well as Oct4 and Nanog [64] in head and neck CSCs. Silencing of BMI-1 subsequently increases the sensitivity of HNSCC cells to chemotherapy and radiation [75]. Recent analysis has indicated that a number of pathways are upregulated in HNSCC following cisplatin treatment, including TNFα, IFN, IL-6/STAT, and NF-κB [76]. Work in other tumor histologies has also supported a phenotypic shift of cancer cells induced following chemotherapy [77].

CSCs in the tumor microenvironment

Finally, emerging evidence supports the theory that the local environment plays an important role in the behavioral governance and regulation of stem cells [78], including the propensity to migrate and adapt to new local environments, forming metastases. This process is generally referred to as the epithelial to mesenchymal transition (EMT) and may endow cancer cells with a stem cell phenotype [79]. This EMT process is governed by transcription factors Snail and Twist [80]. The increased expression of Twist results in decreased expression of the adherence molecule E-cadherin and the resultant propensity to migrate [81]. Twist is also upregulated by the hypoxia-related HIF1 in the

induction of metastases [82]. Following migration, the CSCs are dependent on factors produced in the niche to maintain their survival and stem cell-like state. Gene expression analysis has shown that endothelial cells upregulate IL-6, CXCL8 (IL-8), and EGF when co-cultured with HNSCC cells [83]. When endothelial cells are selectively ablated, the proportion of cancer stem cells in HNSCC xenograft tumors decreases [65]. Given their augmented tumorigenic potential and propensity to form metastases, CSCs are an appealing target for translational research. The disrupting the CSC niche provides a potential opportunity for therapeutic intervention.

CSC in vitro modeling techniques

Several different methods have been used for the isolation of head and neck squamous cell carcinoma CSCs. A number of putative CSC markers compatible with antibody or enzymatic detection have been defined, including elevated aldehyde dehydrogenase-1(ALDH1) [69, 70], CD44 [52], and CD133 expressions [84, 85] on cancer cells. The CSC phenotype can be improved using combinations of these markers [65]. Each of these markers has fluorescence-assisted cell sorting (FACS)-compatible antibodies or assays available, making the identification of HNSCC CSCs via FACS convenient. The side population (SP) assay can identify CSCs via FACS based on the identification of functional characteristics. CSCs are known to be resistant to chemotherapy and are hypothesized to have differentially increased efflux toxin pumps compared to non-CSCs. Side population cells are identified based on their ability to eliminate the nucleic acid stain Hoechst 33342, which is fluorescent when bound to double-stranded DNA [86, 87]. Side population cells have been identified and characterized and also express augmented stem cell marker expression [72, 88–90].

Other culture-based assays for HNSCC CSC identification also rely on the functional characteristics. Cancer stem cells including HNSCC are able to grow in an anoikis-independent manner, and thereby evade apoptotic signaling from loss of extracellular membrane contact [91–95]. Anchorage-independent growth can be leveraged to isolate CSCs by designing culture environments with low or absent cellular attachment. While protocols differ by type of cancer, putative CSCs grown in cell culture dishes designed to minimize attachment and fed with media with selected growth factors, but in the absence of serum, enriches spheres of CSCs [94, 96, 97] (Fig. 2b). Head and neck cancer-specific orospheres and their respective isolation protocols have been identified [65, 98]. To further improve the quality of CSC isolation by minimizing attachment, recent reports have included tumor sphere isolation in both hanging drops [99] and non-attachment ware systems [62].

To identify and manipulate the cancer stem cell population, a source for tumor cells is required. Immortalized cancer cell lines have been developed in a variety of cancer types, including HNSCC [100]. For translational experiments, cell lines are implanted in immune suppressed mice to form tumors, but the therapeutic responses of these xenograft tumors does not always correspond with results from human trials [101]. Numerous HNSCC cell lines have been characterized [102] (Fig. 2a). To improve the concordance between laboratory experiments and clinical data, patient-derived xenografts (PDX) were developed with hopes of maintaining an in vivo tumor model with a closer relationship to the original tissue [103–105]. PDX models are now considered the gold standard for pre-clinical trials for testing of new therapies in vivo (Fig. 2c).

In silico CSC modeling techniques

As the approaches above imply, there are numerous technical hurdles that must be overcome to study a rare cellular population like the CSC pool. As computing resources have become faster and more accessible, mathematical models have become increasingly appealing to help extrapolate and explain data from experimental studies in the larger context of tumor growth dynamics [106–108]. The literature on cancer stem cell modeling is vast, and we will highlight the differences between approaches here without delving deeply into technical aspects of the models, with specific highlights for models that have been used to model head and neck cancer stem cells (Table 1).

Stochastic probability theory has given rise to a number of different modeling tools, including Markov chains. Markov chains are appealing in their ability to generate a unique long-term stationary equilibrium distribution independent of the starting state [109–111]. Markov chains require the restrictive assumption that different cell states all have equal growth rates [112], which limits their utility in realistically estimating the cell numbers. However, other stochastic process-based methods have been developed to model cancer stem cell growth and resistance [113]. The probabilities for extinction of a given subgroup have been modeled using birth/death processes [114, 115]. Multistate branching processes have been deployed to model cellular hierarchies, such as the relationships between cancer stem cells and non-stem cells [116–118]. Explicit application of branching process theory to real-world data can be limited by formal requirements on the composition of the transition matrix [118]. Some feedback between cellular states is likely required to reach long-term equilibrium in stochastic models [119–121]. In one stochastic modeling application,

Table 1 Advantages and disadvantages of selected cancer stem cell in silico modeling techniques

Modeling technique	Advantages	Disadvantages
Markov	Establishes the steady-state proportion of the given cell types	Requires cell types to grow at equal rates
Stochastic processes (birth/death and branching)	Estimates both cell number and proportions	Requires feedback between states to reach a stable steady state
Differential equation based (partial and ordinary)	Flexible, deployable to address a large number of questions	Requires numerous parameters, some of which might not be measurable
Agent-based models and cellular automaton	Details of three-dimensional growth of tumors composed of individual cells	Computationally expensive, complex to construct, assumption dependent

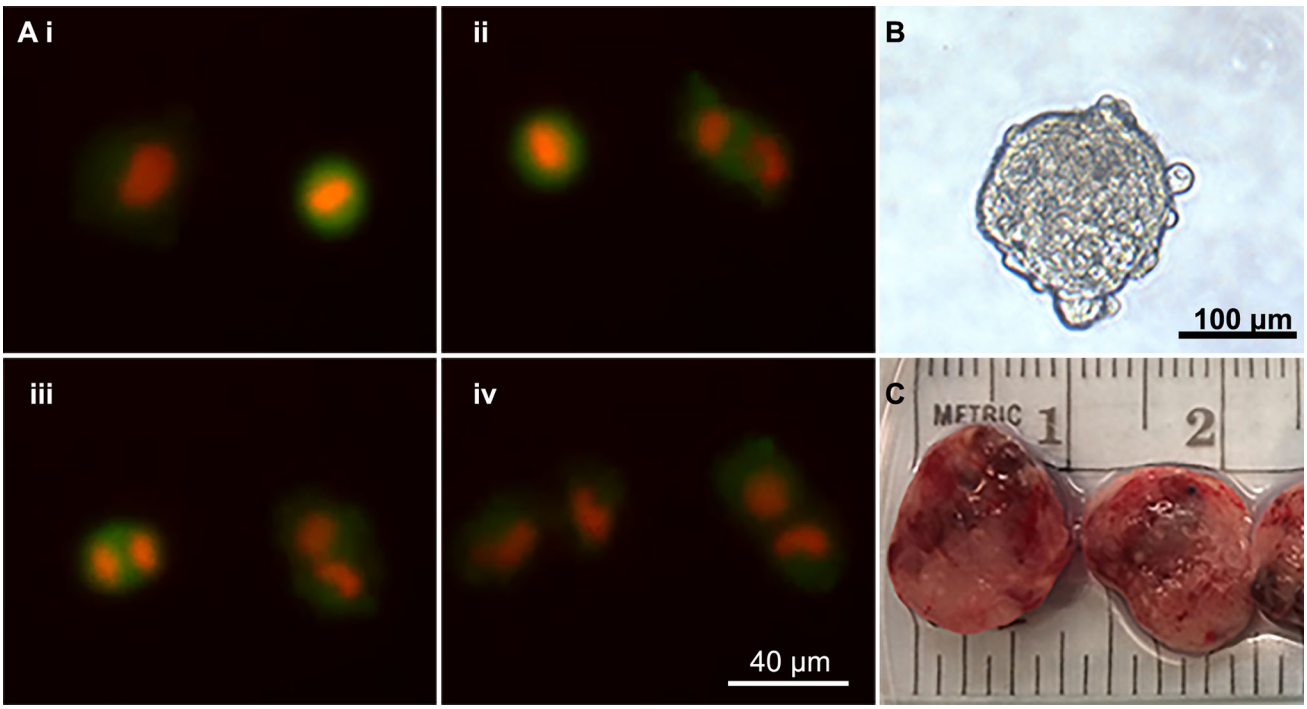

Fig. 2 In vitro and in vivo modeling: **a i–iv** Real-time fluorescent microscopy of head and neck squamous cell carcinoma cells undergoing mitosis in standard cell culture with red (nuclear) and green (cytoplasm) fluorescent reporters. **b** HNSCC sphere grown in sphere media. **c** Patient-derived xenograft tumors grown and excised from murine hosts

in vitro experiments were used to validate unexpected model predictions that indicated isolated populations of breast cancer stem cells pass through an intermediate cell subtype before settling into a stable resting distribution [112]. The output from stochastic models can often express the relative probabilities of different results classes (Fig. 3a).

In parallel to stochastic process approaches, a wide variety of deterministic mathematical approaches have also been developed to model cancer stem cells. In contrast to stochastic models, deterministic models generally offer more flexible options for defining the growth and proportional changes between their cell states. One unifying component of modeling techniques specific for stem cells is the assumption that stem cells are a distinct subpopulation of cells, and that the transition or division hierarchies between cells can be defined. The defined hierarchies between cell subtypes have also been used to model the pattern of mutations leading a cell from normal to pre-malignant to carcinoma [122, 123] and the initiation of tumors by CSCs [124]. When the goal is to model tumor size changes or CSC proportion differences, discrete methods [125], ordinary differential equations [122, 126, 127], and partial [128, 129] differential equation networks have all been employed. The flexibility of differential equation-based models is balanced by the complexity of the resultant equation networks and the number of parameter values required for simulation experiments. Mathematical models output can vary widely based on small variations of some parameters, or parameters may not

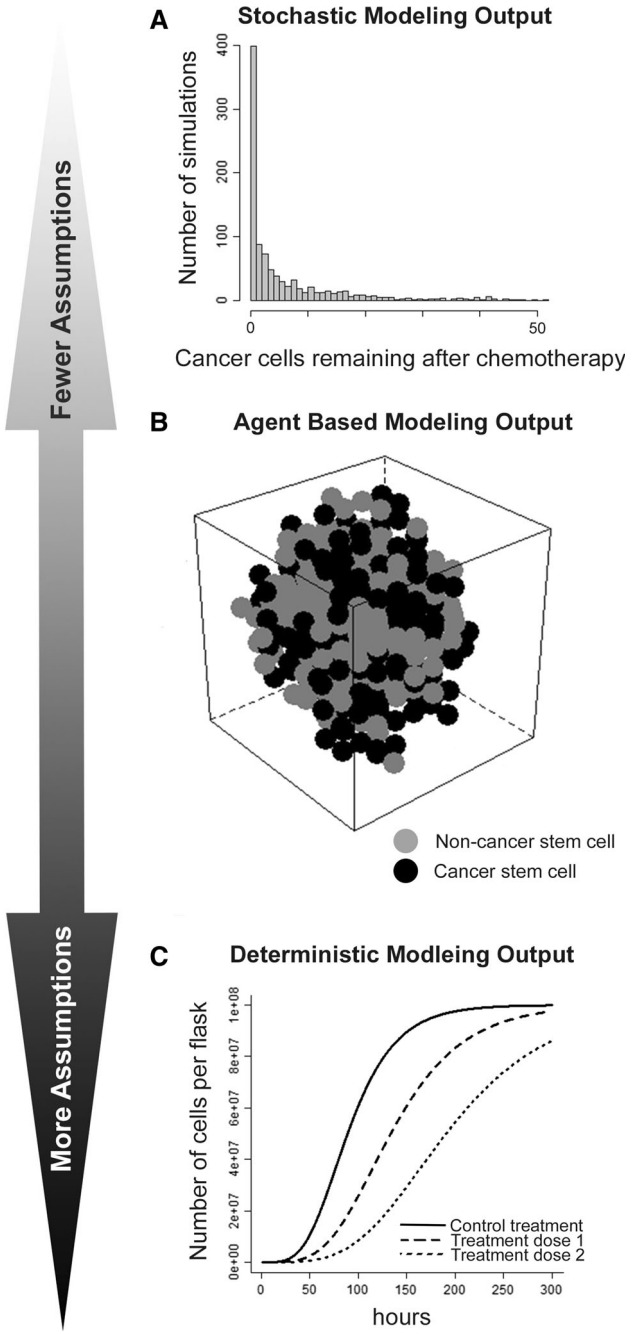

Fig. 3 Different modeling schematics require different levels of assumptions for output: Here, we illustrate the output for three general modeling approaches. **a** Example of stochastic modeling output for a model simulating the number of simulated cancer cells persisting following administration of chemotherapy. **b** Example of ABM output for a simulated tumor consisting of both stem cells and non-stem cells. **c** Deterministic modeling output illustrating the number of cells in a tissue culture media over time with different treatment dosages applied

be observable by experimental means. Deterministic models have been used to investigate a wide range of cancer stem cell-driven tumor growth dynamics and have shown that genetic increases in the number of mutations, particularly in stem cells, leads to a more rapid rise in cancerous cell population than increases in the cellular growth rate or decreases in the cellular death rate [126]. Deterministic models can be helpful for visualizing the estimated relationships over a range of variable values (Fig. 3c).

A most recent area of advancement in mathematical and computational approaches includes hybrid and multiscale cellular automaton and agent-based models. These methods share the approach of assigning behavioral constraints to a number of objects within an in silico environment with defined rules. Simulations can then provide visualization outcomes of different environmental conditions or treatments within a multidimensional environment [130]. Newer hybrid cellular automaton models incorporate the responses to additional non-local elements (such as a treatment or signal) as part of the computational simulation [131, 132] and include the three-dimensional shape of a tumor as part of the output [133, 134]. Multiscale hybrid cellular automaton models can also be formulated to allow phenotypic evolution of cells within the model to more richly replicate the tumor environment in silico [135]. Software packages have been developed to allow researchers to deploy simple cellular automaton models without significant programming expertise, but more complex cellular automaton and agent-based models still require programming proficiency and can be time consuming as computer processors predict a multitude of dependent interactions. Agent-based cellular automaton models are able to explore a wide range of spatio-temporal tumor growth dynamics and have shown that more phenotypically homogeneous tumors grow in a more regular spherical pattern [133]. Unique to agent-based models and cellular automaton programming is the production of a full tumor representation that can be visually inspected for its characteristics (Fig. 3b).

Head and neck cancer offers unique opportunities for modeling advancement, given the combination of complex three-dimensional environment, field risk effect, viral infection component, and multimodality treatment. These opportunities have not translated into a large number of advancements in head and neck cancer modeling. Recently, mathematical models were developed to describe complex tissue shapes to help describe the field effect [136], but they have not been deployed specifically on head and neck data. The role of random chance in the elimination of HPV in squamous cell carcinomas was modeled in relation to CSCs [137]. Models have also been developed with HNSCC data to describe the interaction between head and neck carcinoma cells and tumor endothelial cells under different treatment conditions [138–140].

In silico models of CSCs are an emerging means to explain complex phenomena, yet have also generated a

number of intriguing hypotheses. Combinations of mathematical results show that the CSC fraction will continue to increase over time until CSCs encompass 100 % of the tumor population [133, 134, 141]. Additionally, agent-based models have shown that therapeutic treatment of tumors with chemotherapy or radiation could uncover a new aggressive, CSC-enriched tumor population [142]. Finally, one research group integrated modeling into translational research by designing a differential equation network based on interactions of the B-cell lymphoma (BCL) cell death regulating family of proteins [139]. The simulation-based optimal metronomic dosing design was validated in a series of laboratory studies [143] and resulted in a clinical trial (ClinicalTrials.gov ID: NCT01285635).

Conclusions and future directions

Cancer stem cells are an emerging culprit for pathogenesis in head and neck cancers. While no single molecular profile predominates, CSCs share the ability to form tumors with few initiating cells and evade traditional cytotoxic chemotherapy approaches for eradication. To more efficiently study this important cellular sub-population, several specific in vivo and in vitro methods have been developed for CSC isolation. The complexity of the data produced by a translational laboratory has increased dramatically in recent years. This offers an opportunity for computational modeling to bridge the gap between the significant complexity within true biological systems and the measurable outputs within the laboratory setting. A number of innovative approaches which merge laboratory data and mathematics have been investigated, but head and neck cancer has seen few disease-specific models.

A current area of interest for both CSC and non-CSC oriented research are the phenotypic changes induced in tumors following chemotherapy. Because the ability of computational approaches represent potentially very small cell populations (such as cancer stem cells), mathematical models are a useful tool to describe how therapy changes the tumor milieu. Ultimately, an understanding of how the metastatic niche and treatment factors modify the behavior of head and neck cancer stem cells will enable a rational prioritization of therapeutic investigation.

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