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Cancer Stem Cells, the Tipping Point: Minority Rules?

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Abstract
Purpose of Review Putative studies continue to support the assertion of the cancer stem cell (CSC) hypothesis, namely that a very small subgroup of a malignant tumor population initiates and drives tumor growth. These cells are purported to possess similar biological properties to their normal adult stem cell counterparts. The CSC hypothesis arises from the observation that tumors like normal tissues have their origin in cells that display potential for self-renewal as well as the ability to generate differentiated cells of various lineages. In addition, CSCs have developed basic characteristics that enable them to evade the effects of standard therapies and these may in fact underlie the mechanisms leading to chemo-resistance and tumor relapse.
Recent Findings In recent years, mathematical and computational modeling have emerged as powerful tools in biomedical research that can be used to study biological systems at multiple scales ranging from molecular processes to cell-cell interactions and how these interactions lead to changes at tissue and organ levels. In addition to accelerating biomedical research through computational simulation of physical experiments, modeling can also be used to guide experimentalists by identifying possible factors and mechanisms underlying the particular problem being studied; this in turn may suggest physical experiments that eventually lead to the resolution of this very problem.
Summary In this paper, we review mathematical models that explore the role of CSCs in treatment response, in developing chemo and radio resistance, as well as those that suggest new treatment strategies. In addition, mathematical models that focus on optimal therapeutic protocols will also be discussed.

Keywords Cancer stem cells · Heterogeneity · Mathematical models · Treatment response · Resistance · Targeted therapy

Scope of this Review

Cancer is a group of diseases that involves abnormal cell proliferation in which the interaction of cellular mechanisms and the tumor microenvironment imbue some tumor cells with metastatic potential resulting in the dissemination of malignant cells to other parts of the body. Tumor heterogeneity is one of the important features that has been observed in different types of cancers, and this has a significant impact on tumor development and response to treatment. Both clonal evolution and the CSC hypothesis go some way to explaining the genesis and evolution of this heterogeneity [1, 2]. Cancer clonal evolutionary theory suggests that tumor initiation relies on multiple mutations occurring in an arbitrary single cell [3]. However, the cancer stem cell hypothesis proposes that a small sub-population of cells, known as cancer stem cells (CSCs), are endowed with tumor initiation and propagation potential (Fig. 1). These CSCs are able to perpetuate themselves through self-renewal and to generate non-CSC progenies through symmetrical and asymmetrical divisions, respectively. Recent evidence suggests that the transition from CSCs to normal cancer cells is not unidirectional, and that there is a degree of plasticity between non-CSC and CSC states [4, 5].

This article is part of the Topical Collection on *Mathematical Models of Stem Cell Behavior*

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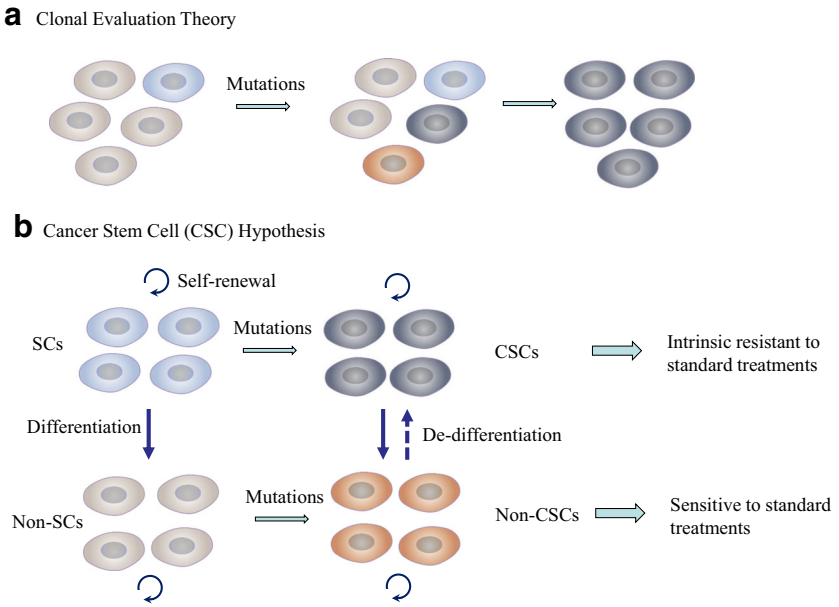

Fig. 1 Schematic diagram of CSC hypothesis and clonal evolution theory. In cancer evolution theory, the acquisition of mutations occurs, followed by expansion of the dominant clone (**a**). But, the CSC hypothesis suggests that CSCs share similar properties to normal stem cells (SCs) and are responsible for cancer initiation as well as the generation of non-CSCs (**b**)

Such interconversion can arise as a result of genetic modifications to cancer cells, random mutations, or microenvironmental effects.

The therapies that patients receive are usually proposed based on the type, the stage and location of a particular cancerous malignancy, and on the overall health of the individuals. The most common types of therapeutic interventions are surgery, chemotherapy, and radiotherapy, and in practice, a combination of more than one treatment is applied. Chemotherapy drugs can target tumor cells in different ways. Generally, these drugs prevent cancer cells from growing and reproducing rapidly through DNA damage; however, in the process, this often results in damage to normal cells, as well. Chemotherapy can be given before, during, and after radiotherapy (referred to as neoadjuvant, concurrent, and adjuvant, respectively). Current conventional radiotherapies also deliver high-energy beams to tumor tissues, which induce various types of DNA damage and genomic instability. Some of the resulting types of lesions, such as double-strand breaks, are severe enough to cause cell apoptosis. However, the majority of treatment failures for different types of cancers are a direct result of the emergent resistance of cancer cells to conventional therapies, which leaves patients with limited treatment options [6, 7]. CSCs show higher resistance to available therapies due to upregulated DNA repair mechanisms. The ability of non-CSCs to reenter the CSC state can also contribute to poor clinical results. Furthermore, most available treatment strategies also target cells that are actively dividing, which is not the most efficient way to destroy CSCs since they are relatively quiescent. In addition, dysregulated signaling pathways that control CSC self-renewals, including Notch, PTEN, BMI-1, and WNT, are usually not targeted by current conventional therapies [8]. Thus, understanding the tumorigenic potentials and the effective mechanisms that CSCs develop to enhance their aggressive phenotype is essential for the development of more efficient and effective treatment strategies.

Along with the concerted effort that is underway in different branches of science to combat cancer, mathematical models have also been effectively utilized to probe the underlying mechanisms driving tumor growth and make predictions that can be validated experimentally [9–12]. For example, mathematical modeling that is grounded in experimental data can be used to predict therapeutic outcomes and improve clinical results [10, 13–16]. Mathematical modeling has been extensively used to try and understand cancer on different scales, but in this review, particularly, we focus on models that try to simulate and predict the effects of treatment. Hence, a brief review of these types of models will be given in the introduction. The main scope of this article is to highlight various mathematical models that incorporate the CSC hypothesis as well as some of the fundamental traits of CSCs. These will be discussed in more details in Sections 1, 2, and 3.

Introduction

Earlier seminal work of Norton and Simon [17, 18] utilized mathematical modeling to integrate biological growth information in to treatment scheduling. This led to perhaps the greatest clinical trial innovation in half a century and the wide spread acceptance of the "Norton-Simon" hypothesis in clinical circles. Norton and Simon proposed that tumor growth dynamics follows a sigmoidal function during chemotherapy and suggested that a dense dose protocol would have better outcomes than standard schedules, which has been clinically verified, for example in [19] among numerous others. In addition, a number of

mathematical models discussed tissue response to fractionated radiotherapy treatments with either acute or protracted doses [20–22]. One of the early models in this area was developed by Thames et al. [23], Thames [24] who used it to investigate the dynamics of radiation damage repair. The approach used a linear quadratic model to describe cell survival, modified to account for incomplete repairs between fractions (for fractionated acute continuous exposure) and the repair during the administration of the fractions (for low dose rate continuous exposure). The linear quadratic model and its modifications have been considered extensively in the literature, to simulate the response to radiation exposure. The evolution of resistance before and during treatment is also one of the first problems that was addressed in the mathematical modeling of treatment responses [25–28]. Coldman and Goldie [25] and Goldie and Coldman [26] proposed a stochastic model to explore the risk of developing resistance during treatment. The model assumed that sensitive cells can be eliminated upon receiving treatment and that resistant mutations can occur with a certain given probability. The results imply that the probability of resistance (when treatment includes two drugs given sequentially) depends on the total number of cells and their mutation rates. The authors suggested that to improve success rates, drugs should be administered as soon as possible after diagnosis. They proposed that drugs should be given in an alternating fashion rather than sequentially to have a significant impact on the heterogeneous cell populations; nevertheless, this suggestion could not be confirmed clinically [29].

In medicine, to proceed from bench to bedside, numerous clinical trials are needed to determine the best treatment procedure and protocol. In this context, mathematical modeling can play an important and critical role in the prediction of the most efficient treatment strategies, thus avoiding unnecessary and often excessive reliance on clinical trials. Several mathematical models have been developed in the literature to establish the most practical treatment protocols [30–32]. Many models try to rapidly minimize the total tumor size; however, successfully controlling tumor growth depends critically on reducing effectively both drug sensitive and drug resistant cells. Some of the early work by Costa et al. [33] describes the dynamics of a tumor that includes drug resistant cells. The model aims to efficiently find the optimal treatment schedule by minimizing the total tumor size. The development of better-designed treatment regimens is still a field of significant research activity; nevertheless, the attainable benefits from treatment must still be evaluated and quantified to be of any clinically relevant significance. The tumor control probability (TCP) is a measure that attempts to quantify the probability of destroying or removing malignant cells using a variety of radiation therapy schedules. In order to establish a better formalism for TCP, different models have been introduced in the literature such as that of Kendal [34], Munro and Gilbert [35], Tucker et al. [36], Yakovlev [37], and Zaider and Minerbo [38]. For example, one model frequently discussed in the radiation therapy literature is the so-called Poisson model of TCP [35, 38]. This model assumes that the number of cells that survive radiation has a binomial distribution, and if the survival probability is small enough, the probability of no malignant cells remaining follows a Poisson distribution after treatment. However, the model neither captures the proliferation of cells during treatment nor the stochastic effects. Later, Zaider and Minerbo [38] acknowledged the impact of stochastic effects on radiation-induced cell death and suggested a model based on a simple stochastic birth/death process.

Generally, most of the primary mathematical models are established based on the clonal evolution theory, where all cells are capable of giving rise to mutants that lead ultimately to the formation of tumors. However, the emerging CSC hypothesis has become the subject of theoretical analysis to explore the role of CSCs in tumor response to treatment and the acquisition of resistance, which will be discussed in the following sections.

The Roles of CSCs in Evolving Resistance and Tumors Response

Despite much improvement in the design of practical cancer therapies, the majority of patients often develop tumor resistant to standard therapies [39, 40•, 41, 42]. It may be that conventional cancer treatments act more efficiently on highly proliferating cells and thus leave the quiescent CSCs relatively unscathed. For example, resistance to imatinib is one of the complications that can arise for patients treated for chronic myelogenous leukemias (CML), which can cause initial refractoriness of the disease and relapse. The evolution of resistance from an exponentially growing cell population was studied using a continuous time branching process by Iwasa et al. in [43]. The model starts with a single sensitive cell that can undergo mutations and become resistant to imatinib. Finally, the probability of resistance at the time of diagnosis was calculated and it was concluded that a higher number of cell divisions increase the occurrence of resistant cells. The quiescence of cancer stem cells is also a critical characteristic that safeguards them from imatinib. Hence, a mathematical model has been developed to explore the impact of cellular quiescence on the dynamics of drug resistance [44]. For a single drug, if the resistant cells exist before treatment, the quiescent cells do not modify the chance of resistance, although they can increase the probability of developing resistant mutants when patients receive a combination of more than one drug with various targets. In fact, the therapy phase is not important for emergence of mutants since they existed before the diagnosis, but the dormant cells may delay the time that is required for the therapy to eliminate the tumor burden. The authors ultimately suggested that reducing the number of quiescent stem cells during therapy is not beneficial for reduction of

resistance risk, since plasticity is another key factor that contributes to resistance and invasion. Poleszczuk et al. [45] used a mathematical model to simulate and investigate the effects of different rates of transitions (from non-CSCs to CSCs) on tumor growth and treatment response. The results show that tumors with low rates of plasticity can regrow after radiotherapy. Nevertheless, for tumors with high plasticity rates, post-therapy cancerous cells undergo remission after regrowth, because radiotherapy appears to increase CSC depletion. Although the results seem interesting, more experimental investigation is required, to ensure this is not a computational artifact.

Gupta et al. [4] combines both biological experiments and mathematical simulations to examine the sensitivity of distinct phenotypic states (Stem like, basal, luminal) to treatment. For this purpose, breast cancer cell lines (SUM159 and SUM149) have been treated with two conventional chemotherapy drugs: paclitaxel and 5-fluorouracil (5-FU), which resulted in increasing the portion of cancer stem-like cells for both cell lines. To gain a comprehensive understanding of these results, a Markov model has been established to examine the dynamics of breast cancer cell populations and transition between different states. The results indicate that basal cells are more sensitive to paclitaxel in comparison to the other two states for the SUM159 line. Moreover, the proportion of both stem-like cells and basal cells show approximately a fivefold increase after receiving paclitaxel, but the growth in basal cells is due to the resistance of stem-like cells to the treatment, which can regenerate basal cells afterwards. In addition, Gao et al. [46] demonstrate that resistance to radiotherapy is not the only critical factor responsible for CSC enrichment in gliomas and that repeated exposure to radiotherapy can create a microenvironment that tilts the proliferation in favor of symmetric divisions.

Cell surface protein expression profiles are the main tool used to isolate cancer stem cells in different tissues. For example, $CD34^{high}CD38^{low}$, $CD133^{+}$, and $CD44^{high}CD24^{low}$ are common biomarkers used for leukemia and brain and breast tumors, respectively, see [47] and references there in. However, clearly not all cells that have the same protein expression are necessarily cancer stem cells, and it appears that both cancer stem cells and early generations of progenitors often express the same protein markers [48]. These findings have been taken into consideration in developing a hierarchical model that includes stem cells, the N^{th} generation of progenitor cells, and mature cells. Dhawan et al. [14] have employed a fully stochastic model for a hierarchy of heterogeneous cell populations and used numerical simulations to obtain the tumor control probability (TCP). The TCP is defined as the probability of eradicating all cancerous cells in a particular tissue and is used as a measure of radiotherapy efficacy. Based on the CSC hypothesis, removing CSCs is essential to achieve a cure. Therefore, the probability of controlling cancer stem cells only (TCP_s) was also determined. Furthermore, because of imperfect biomarkers for CSCs, the probability of eliminating biomarker positive cells (TCP_{CD^+}) was calculated. Finally, it is suggested that TCP_{CD^+} can be used as a clinically relevant alternative for TCP_s.

New Therapeutic Strategies Targeting CSCs

CSCs are generally not targeted by commonly used treatment strategies, so designing therapies that are able to specifically target CSCs is of paramount importance [49••, 50, 51]. For example, it has been shown that the fraction of CSCs is enriched after radiotherapy due to the highly efficient DNA damage response in gliomas [39]. Thus, developing effective treatment strategies that target and eradicate CSCs is crucial to improving clinical results and minimizing recurrence. Consequently, designing therapies that include both standard anticancer treatments and CSC-targeting agents may be an effective double-pronged attack to eliminate various types of cancer cells. For instance, Goldman et al. [52••] used both mathematical modeling and experimental studies to investigate the mechanisms behind adaptive resistance in breast cancer patients treated with a high concentration of taxanes. The results indicate that treatment with taxane leads to a phenotypic cell state transition to the CSC population, which can contribute to tumor resistance. Moreover, it is demonstrated that applying inhibitors that can control the SFK/HcK pathways in a proper temporal schedule (after exposure to taxanes) increases the sensitivity to chemotherapy treatment and thus increases cell death. Furthermore, a simple mathematical model has been presented [53] to illustrate the importance of eradicating CSCs. The model includes two layers of differential equations to account for the hierarchy of stem cells and differentiated cells for both normal and tumor cells. Analysis of different therapeutic possibilities implies that increasing apoptosis or decreasing the generation of malignant mature cells are not useful approaches to controlling and removing the disease due to plasticity and replenishment of CSCs. However, the therapeutic protocols that prevent CSCs from reproduction have the potential to eradicate the disease if CSCs are subjected to such a therapy for an extended period of time. Furthermore, it is predicted that agents that either decrease the division rate or increase the death rate of CSCs can improve the results; however, the eradication of cancerous mature cells is needed to minimize the risk of failure and eliminate the potential impact of plasticity. Additionally, a mathematical model has been developed based on the work of Youssefpour et al. [54] to explore the advantage of "differentiated" therapies and radiotherapy combinations, which push CSCs to differentiate into descendants that are more sensitive to radiotherapy [55]. Consequently, applying "differentiated" therapies along with radiotherapy appears to

improve treatment success and decrease side effects for head and neck, brain, and breast cancers.

Piccirillo et al. [56] have reported that exposure to bone morphogenetic proteins (BMPs) decreases proliferation and increases the expression of non-cancer initiating cells in glioblastomas (GBMs). This study demonstrated that brain tumor stem cells (BTSCs), identified by biomarker $CD133^+$, are induced by BMPs to differentiate into $CD133^-$ cells, which are not tumourigenic and are more responsive to conventional cancer therapies. These findings suggest that adding proteins like BMPs to the currently available radiotherapy protocols might significantly improve outcomes; nevertheless, more investigation is required due to other possible interactions in the complicated underlying mechanisms driving tumor growth. From this perspective, Turner et al. [57] have proposed a mathematical model that represents the effect of BMPs on radiotherapy results for glioblastoma based on the cancer stem cell hypothesis. The model describes the stochastic effects of the small number of cells for different types of BTSC divisions, symmetric self-renewal $S \rightarrow S+S$, asymmetric self-renewal $S \rightarrow S+P$, and symmetric proliferation $S \rightarrow P+P$. These two subgroups of cells can also undergo apoptosis and be discarded. On a larger scale, however, the model considers the corresponding average equation to study the role of BMPs and the cell kill response of radiotherapy on tumor dynamics. The model is mathematically given by:

$$\frac{dS(t)}{dt} = \tilde{\rho}_s(S,P) r S - \Gamma_s S - \alpha_s S \sum_j d_j f\left(\frac{t-t_j}{\tau_s}\right)$$

$$\frac{dP(t)}{dt} = \tilde{\rho}_p(S,P)(1-r) S - \Gamma_p P - \alpha_p P \sum_j d_j f\left(\frac{t-t_j}{\tau_p}\right)$$

where $\tilde{\rho}_p(S,P) = \rho_s\left(1-\frac{S}{S_{lim}}-\frac{S}{S_{lim}}\right)$, which employs logistic growth dynamics to capture the competition between species for limited nutrition. Here, S_{lim} and P_{lim} stand for the maximum population of BTSCs and progenitors, respectively. Additionally, ρ_s denotes a rate of proliferation for stem cells that can occur with probability $r = r_1 - r_3$, where $r_1$and r_3 are the probability that BTSCs go through symmetric self-renewal and symmetric proliferation, accordingly. These two types of cells can undergo apoptosis with probability Γ_i ($i \in \{S,P\}$). In addition to apoptosis, cells can also be removed with radiation dose d_j given at time t_j on j^{th} fraction of treatment. Here, the function f is assumed to be exponential for x≥0 and 0 otherwise. The clearance times for dead BTSCs and progenitors after radiation are given by τ_s and τ_p, respectively. Further, α_i for $i \in \{S,P\}$ represents the radiobiological parameters for BTSCs and progenitors. Experimental results demonstrate that BTSCs are more resistant to radiation than $CD133^-$ cells [39]. Therefore, the radiosensitivity parameter for $CD133^-$ cells is chosen to be 3 fold more than the radiosensitivity parameter for $CD133^+$cells ($\alpha_s < \alpha_p$).

The effect of BMPs is mathematically captured by reducing the probability r together with fixing r_2, which is the probability of BTSCs going through asymmetric self-renewal. Following Piccirillo et al. [56], it is assumed that $r = r_1 - r_3$ is changed from the pretreatment value 0.1 to a negative value −0.1 after receiving BMPs. Modifying r to a negative value implies an increase in symmetric differentiation divisions and a decrease in symmetric self-renewing divisions. The effect of radiation kill is also examined for different treatment schedules. Since the model assumes a higher radiosensitivity for $CD133^-$ cells, the fraction of BTSCs is elevated. In addition, eradicating $CD133^-$ cells raises the number of $CD133^+$ due to the logistic growth impact on cell proliferation that necessitates a small increase in the number of BTSCs after radiotherapy (in comparison with the control group). But, BMP therapy-only lowers the number of BTSCs at the expense of a slight increase in the number of $CD133^-$ cells. Regardless, the results have shown that adding BMPs or probably any other CSC targeting agents in addition to radiation therapy effectively shrinks the tumor along with an associate decrease in $CD133^+$ cells.

Finding the Optimum Treatment Schedule Under the CSC Hypothesis

Cancer treatments have evolved over time with the purpose of enhancing life expectancy for cancer patients. In the last two decades, mathematical modeling has started to play an important and pivotal role in developing optimal treatment strategies and protocols as well as providing a new experimental tool for investigating the impact of a new proposed therapy on tumor cells, in silico [58–61]. For instance, the analysis and simulation of Powathil et al. [61] suggest that the combination of neo-adjuvent chemotherapy followed by radiotherapy might be a better treatment strategy than adjuvant chemotherapy for gliomas. Understanding the importance of targeting CSCs and their distinct properties may lead to the development of new therapeutic protocols, which might achieve better tumor control. As an example, Enderling et al. [15] presented a mathematical model that studies the effect of CSCs and quiescent cells on treatment outcomes. The CSC fraction size and the stem cell proliferation rates have been reported as critical factors determining treatment response. Assuming less radiosensitivity for quiescent cells, which are mainly located in the core of a solid tumor, it has been suggested that applying hypofractionated radiation protocols can control the disease if the CSC pool size is small and as long as the CSC repopulation does not interfere with the higher capacity of radiation kill. Moreover, heterogeneity and instability among various lineages of cancer cells can reduce the potency of available treatment options. Hence, a mathematical model and an experimental study were designed to predict an efficient

radiotherapy regimen for glioblastoma [60]. The model considers plasticity between CSCs and differentiated cells and assumes that CSCs are more radioresistant. Furthermore, surviving cells lapse into a quiescent state after radiotherapy, but can repopulate again, after exiting quiescence. Consequently, two radiotherapy protocols, which deliver larger fractions at the beginning and end of radiotherapy treatment, have been recommended claiming to lead to better outcomes than conventional therapies. These predicted regimens have been tested experimentally and demonstrated to lead to greater survival in mice. The model was later extended to predict a radiotherapy regimen maximizing survival and minimizing toxicity in the corresponding tissues arising from exposure to larger doses of radiation at the beginning and end of the therapy [62]. The problem is reduced to two optimization problems: the first deals with optimization of the total dose and dose per fraction, and the second handles optimization of time intervals for each fraction. The results obtained imply that the best arrangement for the time intervals corresponds to the dose distribution that maximizes the return to the stem-like state. However, these approaches may lead to a growth in CSC population, which can contribute to therapy resistance and recurrence.

Conclusion

The emergence of resistance to conventional therapies has been long recognized as one of the major causes of tumor relapse and recurrence. CSCs, also known as cancer initiating cells, develop superior mechanisms such as activated DNA damage repair, upregulated drug transporters, and maintenance of cellular pathways which allow them to survive standard therapeutic protocols and trigger relapse in many cases. Therefore, identifying and understanding the role of CSCs in therapeutic resistance can improve the overall efficacy of available treatments and assist in the development of new treatment strategies targeting CSCs. Here, mathematical modeling following experimental validation is useful to understand the underlying mechanisms and design new treatment approaches.

In this review paper, we have presented an idiosyncratic survey of mathematical models that investigate the impact of different characteristics of CSCs such as differentiation, quiescence, and plasticity, on treatment response and emergent tumor resistance. However, CSCs employ other complex mechanisms such as upregulated drug transporters, which play critical roles in the development of tumor resistance. Mathematical oncology is a nascent field of research with the potential for significant clinical impact, but this requires much more theoretical investigation using mathematical and computational modeling validated through experimental results. Moreover, studying the impact of microenvironmental effects (e.g., hypoxia) on the proliferation and control of CSCs may lead to significant advances in clinical oncology.

Furthermore, in this article, we have also reviewed mathematical models that provide experimental predictions in the quest to develop new therapeutic strategies targeting CSCs. The main purpose of these new treatment strategies is to increase the sensitivity of CSCs to chemotherapy and radiotherapy. This includes a combination of conventional therapies with molecular inhibitors controlling CSC pathways, which enhance CSC death. Self-renewal is considered to be the main reason for radioresistance in CSCs, but understanding other pathways such as those contributing to apoptosis is also of clinical interest [51]. Here, mathematical modeling can be applied to predict other critical pathways and possible clinical outcomes, which can be validated experimentally. In addition, using chemotherapeutic agents together with radiotherapy to increase the effect of radiation on CSCs have been shown to improve results. However, it is important that these agents inflict minimal damage on normal stem cells since they share many of the same features as CSCs [6, 51].

Current radiotherapy and chemotherapy schedules have been improved in an attempt to optimize treatment outcomes and minimize toxicity. Mathematical models actively play a crucial role in attempts to design better treatment strategies. Nevertheless, most current clinical protocols still focus on reducing the tumor burden and normally disregard CSCs. This can lead to the emergence of resistant CSCs which in turn leads to relapse and aggressive metastatic invasion. Thus, developing mathematical models suggesting new therapeutic schedules that at the same time reduce the fraction of CSCs or include recent molecularly targeted approaches can be helpful. Moreover, clinical and experimental research to improve clinical outcomes are fields that have seen rapid growth in recent years. For example Klement et al. [63] suggested a combination therapy comprised of continuous low dose chemotherapy regimen and a VEGF receptor-2 antibody, to increase the antivascular effects of the treatment in order to shrink the tumor and reduce the evolution of drug resistance. Mathematical and computational approaches herald a new era in clinical oncology with the potential to address questions arising from experimental studies and vice versa to guide experimental studies to resolve many of the puzzles and paradoxes that are part and parcel of cancer biology. Indeed, we are optimistic that these approaches will not only accelerate clinical developments, but elucidate and reveal some of the basic mechanisms driving tumor growth.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
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