Microenvironmental variables need to effect intrinsic phenotypic parameters of cancer stem cells to affect tumourigenicity

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Abstract:

Since the discovery of tumour initiating cells (TICs) in solid tumours, studies focussing on their role in cancer initiation and progression have abounded. The biological interrogation of these cells continues to yield volumes of information on their pro-tumourigenic behaviour, but actionable generalised conclusions have been scarce. Further, new information suggesting a dependence of tumour composition and growth on the microenvironment has yet to be studied theoretically. To address this point, we created a hybrid, discrete/continuous computational cellular automaton model of a generalised stem-cell driven tissue with a simple microenvironment. Using the model we explored the phenotypic traits inherent to the tumour initiating cells and the effect of the microenvironment on tissue growth. We identify the regions in phenotype parameter space where TICs are able to cause a disruption in homeostasis, leading to tissue overgrowth and tumour maintenance. As our parameters and model are non-specific, they could apply to any tissue TIC and do not assume specific genetic mutations. Targeting these phenotypic traits could represent a generalizable therapeutic strategy across cancer types. Further, we find that the microenvironmental variable does not strongly effect the outcomes, suggesting a need for direct feedback from the microenvironment onto stem-cell behaviour in future modelling endeavours.

Introduction:

Heterogeneity among cancer cells within the same patient contributes to tumour growth and evolution. A subpopulation of tumour cells, called Tumour Initiating cells (TICs), or cancer stem cells, has recently been shown to be highly tumourigenic in xenograft models and have some properties of normal stem cells. Although controversial, there is a growing body of evidence that TICs can drive tumour growth and recurrence in many cancers, including, but not limited to, brain [1], breast [2] and colon [3]. These tumour types can be broadly classed as hierarchical tumours as they have been posited to follow some of the same hierarchical organisation as healthy stem-cell (SC) driven tissues. In these hierarchical tumors, TICs can differentiate to produce non-TIC cancer cells or self-renew to promote tumor maintenance. As TICs have been demonstrated to be resistant to a wide variety of therapies including radiation and chemotherapy, the TIC hypothesis has important implications for patient treatments [4]. Specifically, the effect of current strategies on the tumor cell hierarchy should be defined and TIC specific therapies are likely to provide strong benefit for cancer patients.

In a simplified view of the tumour cell hierarchy, TICs can divide symmetrically or asymmetrically to, respectively, produce two TIC daughters or a TIC daughter and a more differentiated progeny [5,6]. More differentiated TIC progeny which still have the capability of cell division are similar to transient amplifying cells (TACs) in the HM and are capable of several rounds of their own symmetric division before the amplified population then differentiates into terminally differentiated cells (TDs) which are incapable of further division. This mode of division and

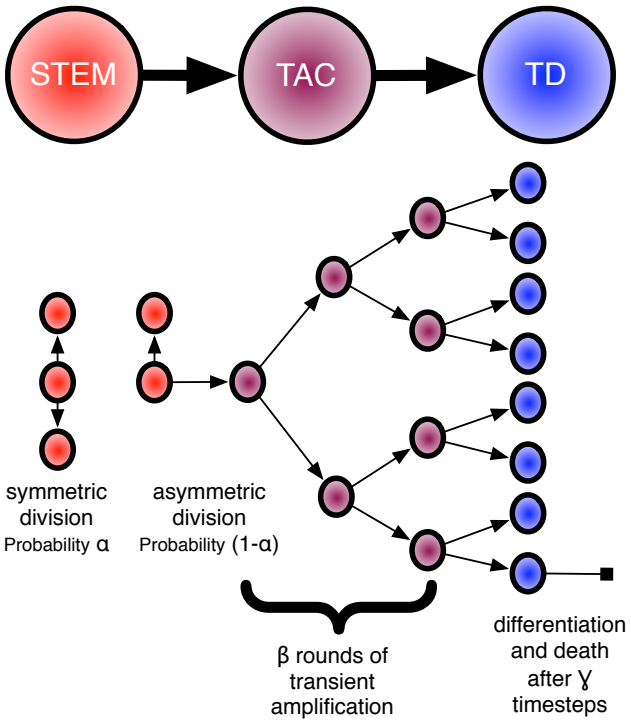

Figure 1. The hierarchical model of stem-cell driven tissues. In this formulation, each stem cell can undergo two types of division, either symmetric (with probability α) or asymmetric (with probability 1-α). Each subsequently generated transient amplifying cell (TAC) can then undergo a certain number (β) of round of amplification before differentiating into a terminally differentiated cell (TD) which will live for a certain amount of time before dying (ɣ timesteps). It is these three parameters, which we assume are intrinsic to a given stem cell, which we explore in this paper.

differentiation, which we will call the Hierarchical Model (HM) can be seen in Figure 1.

In the HM, there are only truly three key cellular behaviours that govern the system. They are the rate of symmetric versus asymmetric division of the stem cells (α), the number of 'rounds' of amplification that transient amplifying cell can undergo before terminal differentiation (β), and the relative lifespan of a terminally differentiated cell (ɣ). While only these three parameters exist, they have been extremely difficult to pin down experimentally and so the majority of the work to describe them has been *in silico*. Most germane to the loss of homeostasis is the work by Enderling et al. [7] which showed the changes to the size of a mutated tissue (tumour) as they varied the number of rounds of amplification of TACs. Other recent work attempting to quantify the ratio of symmetric to asymmetric division in putative glioma stem cells was presented by Lathia et al.[8], who showed that this ratio can change depending on the medium, suggesting yet another method by which a tissue can lose or maintain homeostasis: in reaction to microenvironmental change. A critical limitation of *in vivo* lineage tracing performed to date is an inability to determine the impact of microenvironmental heterogeneity on TIC symmetric division.

While the HM appears to be quite straight forward, there is growing evidence of complexity to be further incorporated into the model. There are likely to be differences in the extent of TIC maintenance or the ability of tumour cells to move toward a TIC state (Fig 3, Right). TICs appear to reside in distinct niches suggesting there may be differences in the biology of these cells, but defining differences in TICs is limited by cell isolation and tumour initiation methods. Prospective isolation of TICs relies on surface markers, including CD133, CD15[1] and CD24 which can be transient in nature [9], due to modulation by the tumour microenvironment or methods of isolation [10]. Characterisation of these sorted cells then requires functional assays including in vitro and in vivo limiting dilution assay as well as determination of expression of stem cell factors including Oct4, Nanog and others [11].

As the importance of TICs becomes more and more evident as it pertains to aspects of tumour progression like heterogeneity [12], treatment resistance [13,14], recurrence [15] and metastasis [16], the need for generalizable therapeutic strategies based on conserved motifs in these cells grows. We therefore aim to understand how the phenotypic traits discussed earlier (asymmetric division rate, allowed rounds of transient amplification and lifespan of terminally differentiated cells) and

microenvironmental changes (modelled as differences in oxygen supply) effect the resultant tissue growth characteristics.

To this end, we present a minimal spatial, hybrid-discrete/continuous mathematical model of a hierarchical SC driven tissue architecture which we have used to explore the intrinsic, phenotypic, factors involved in the growth of TIC driven tumours. We consider parameters that involve the rates of division of the cells involved in the hierarchical cascade as well as micro-environmental factors including space and competition between cell types for oxygen. We present results suggesting that there are discrete regimes in the intrinsic cellular parameter space which allow for disparate growth characteristics of the resulting tumours, specifically: TICs that are incapable of forming tumours, TICs that are capable of forming only small colonies (spheres), and TICs that are capable of forming fully invasive tumours *in silico*, just as we see diversity in biological experiments (Fig 3D).

Methods:

Our model is based on a hybrid, discrete-continuous cellular automaton model (HCA) of a hierarchically structured tissue. HCA models have been used to study cancer progression and evolutionary dynamics since they can integrate biological parameters and produce predictions affecting different spatial and time scales [17-20]. As shown in figure 2C cells are modelled in a discrete fashion on a 500x500 2-D lattice. This comprises approximately 1cm^2 where we assume a cell diameter of 20 micrometers [21]. The domain is assumed to have periodic boundary conditions but simulations are stopped when a cell reaches one of the boundaries. Figure 2A shows that, although all cells are assumed to have the same size and shape, they can only be one of three different phenotypes: SCs capable of infinite divisions, TACs which are capable of division into two daughters for a certain number (β) of generations, and TDs which cannot divide but live and consume nutrients for a specified lifetime (ɣ). Modes of division for SCs include asymmetric division (with probability 1-α), which is division into one SC

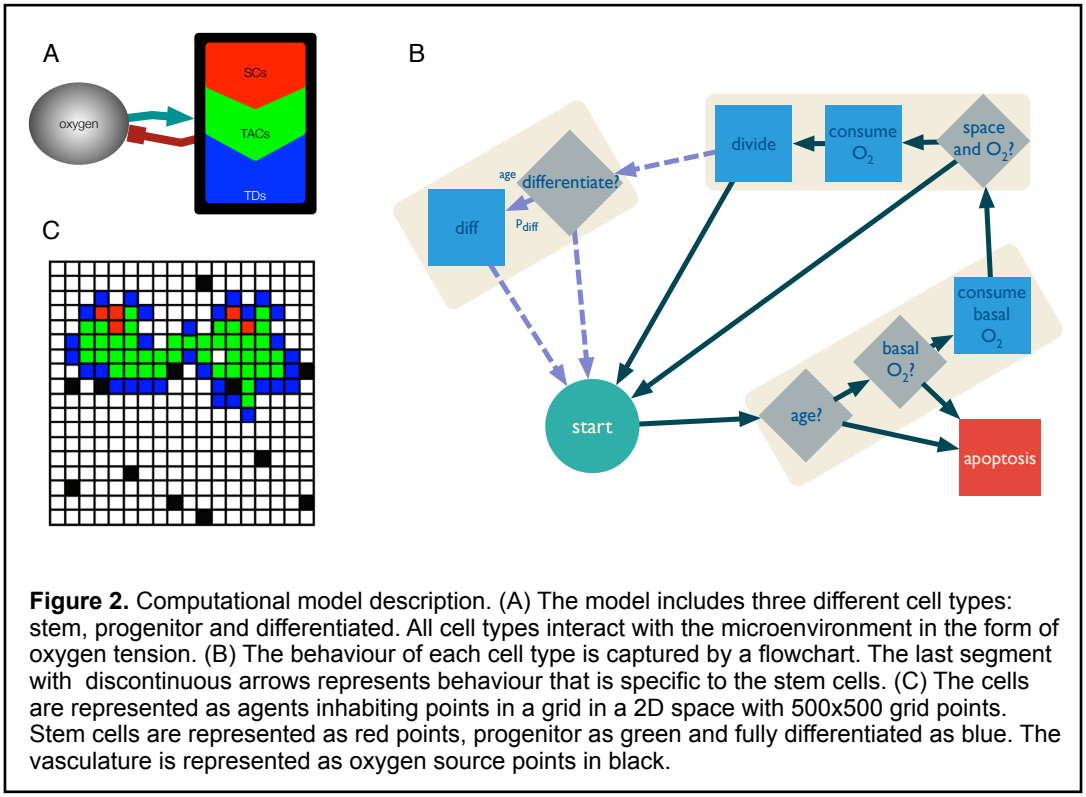

Figure 2. Computational model description. (A) The model includes three different cell types: stem, progenitor and differentiated. All cell types interact with the microenvironment in the form of oxygen tension. (B) The behaviour of each cell type is captured by a flowchart. The last segment with discontinuous arrows represents behaviour that is specific to the stem cells. (C) The cells are represented as agents inhabiting points in a grid in a 2D space with 500x500 grid points. Stem cells are represented as red points, progenitor as green and fully differentiated as blue. The vasculature is represented as oxygen source points in black.

Eq 1. $$\frac{\partial O(i,j,t)}{\partial t} = D_O \nabla^2 O + \lambda V_{i,j} - \mu_S S_{i,j} - \mu_P P_{i,j} - \mu_T T_{i,j}$$

daughter and one TAC daughter and symmetric division, which is division into two SC daughters (probability α).

The continuous portion of this model is made of up the distribution and consumption of nutrients (in this case modelled only as oxygen). Vessels, which are modelled as point sources and take up one lattice point, are placed randomly throughout the grid at the beginning in a specified density (Θ). Each of these vessels supplies oxygen at a constant rate (λ) which then diffuses into the surrounding tissue. The diffusion speed/distance is described by Equation 1, where *O (x,y,t)* is the concentration of oxygen at a given time and place, D_O is the diffusion coefficient of oxygen, λ is the rate of oxygen production from a blood vessel, μ_s, μ_p, and μ_T are the rates at which stem, progenitor and differentiated cells consume oxygen. The difference in time scales that govern the diffusion of nutrients and that at which cells operate is managed by updating the continuous part of the model 100 times per time step. Equation 1 is meant only to illustrate our method concisely, but as the actual implementation is algorithmic, we will not consider it further except to state the condition: $O(i,j,t) \geq 0, \forall t$.

Table 1.
Model parameters.

Parameter	value
D_O (Oxygen diffusion)	0.001728
λ (Rate of Oxygen production)	1
μs, μp, μ_T	0.0001
α (Ratio of SC symmetric division)	0.1, 0.3, 0.5
β (TAC proliferative potential)	1,5,10,11,12,13,14,15,16,17,18,19,20,50,70,100
ɣ (Differentiated cell lifespan)	1
Θ (Vascularisation)	0.001, 0.01, 0.05, 0.1, 0.5

Any simulation performed by this model can be characterised by the parameters found in table 1 the most relevant parameters for the question we are trying to address are the following:

1. Symmetric/asymmetric division rate of stem cells (α)
2. Vascular density in the tissue (Θ)
3. Number of allowed divisions of transient amplifying cells (β)
4. Lifespan of terminally differentiated cells (ɣ)

In each case, as can be seen in figure 3, a simulation is seeded with one TIC with a given set of intrinsic parameters (α, β, ɣ) governing its and its progeny's behaviour, which is placed in the

centre of the computational domain. The domain is initialised with as many randomly placed oxygen source points (vasculature) as described by the vascular density parameter.

Results:

Figure 3 shows examples of the typical results produced by this model. Figure 3a shows an example of an unviable tissue (parameters: 0.001 for vascularisation, a ratio of symmetric vs asymmetric divisions of 0.3, a progenitor replicative potential of 50 and 1 day of lifespan for differentiated cells) where the vascularisation does not support the potential tissue size of that SC, resulting in an area of hypoxia affecting the region that contains the SC. That leads to the death of the stem cell and, eventually, the rest of the cells in the tissue. Figure 3b shows a case of slightly increased symmetric division, resulting in a dynamic homeostasis where cell birth and death is balanced so that tissue size remains relatively constant. Finally, figure 3c shows an example where the system never achieves true homeostasis. In this case the rate of symmetric to asymmetric division is slightly higher when compared with the previous example, suggesting a critical value at which overgrowth occurs. Over time, the number of SCs increases allowing for a larger tissue to be possible. Although this leads to areas of hypoxia, cells survive in the periphery of the blood vessels and keep growing until the take over the entire domain.

A systematic parameter exploration of the three key parameters relating to vascularisation of the domain, symmetric vs. asymmetric division (α) and progenitor division potential (β) was performed. We also explored the parameter determining the lifespan of differentiated cells (ɣ) and found that the only impact of longer lifespans is an increase in the amount of time before the simulations reach a steady state, but does not change the qualitative nature of the results.

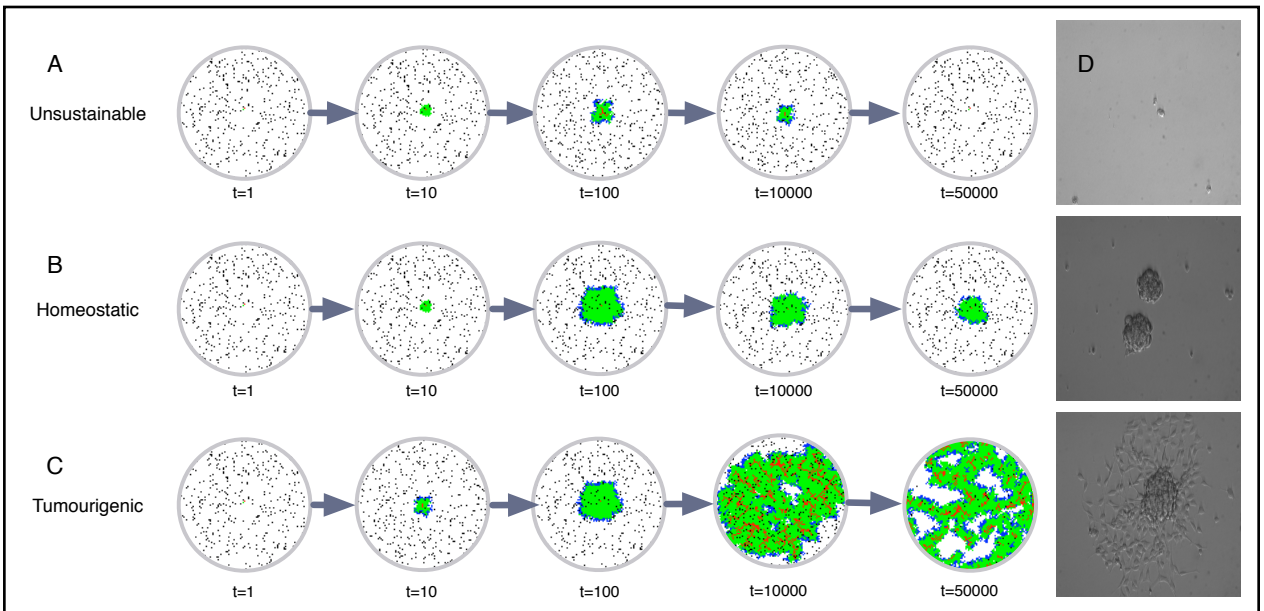

Figure 3. Three different examples of simulations resulting from the computational model. Each simulation represents one of the typical outcomes. Each begins with a single TIC seeded in the middle of the computational domain and the same vascular density. In each situation the phenotype parameters are slightly different, resulting in (A) An unsustainable tissue, (B) A homeostatic tissue where the balance of stem cell sell renewal and progenitor proliferation leads to a tissue whose overall size remains relatively constant over time and, (C) Neoplastic-like tissue where the tissue overgrows the computational domain. (D) Bright field images of clonal CD133+ patient derived glioblastoma cell lines cultured in Neurobasal supplemented with EGF, FGF and B27, exhibiting similar phenotypic variability to the computation model outcomes.

These results are summarised in Figure 4. Each of the three panels represents the results for a different degree of vascularisation (0.01, 0.05 and 0.1). A density of vascularisation of 0.05 would mean 12500 oxygen sources in the domain. To determine the diffusion coefficient, we used the estimate of approximately 70 micrometers of effective oxygenation [22]. Each plot shows the total tissue size after 50,000 time steps as we change the proliferative potential of progenitor cells. Each of the lines shows a different ratio of symmetric vs asymmetric divisions. These results show that all these three parameters have a critical range where homeostasis is disrupted (tumourigenesis).

Unsurprisingly, the higher the vascularisation of the domain the higher the tissue size it can

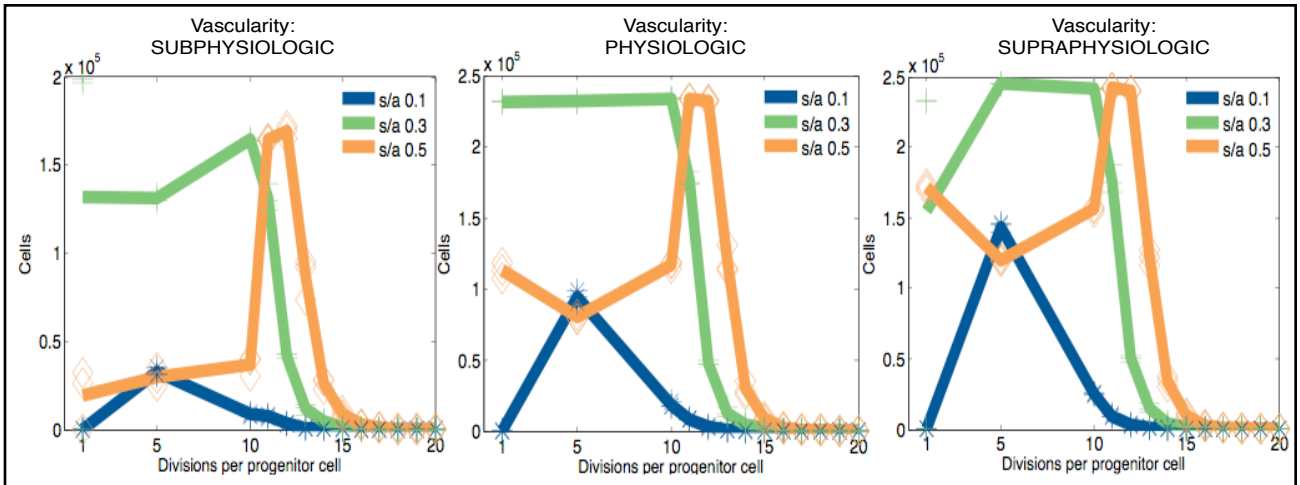

Figure 4. Size of tissues vs. progenitor proliferative potential achieved by simulations using different levels of vascularisation and ratios of symmetric vs asymmetric divisions. (Left). Low vascularisation density of 0.01 (Centre) Normal vascularisation density of 0.05 (Right) High vascularisation density of 0.1. In each of these cases, the maximum tissue size will depend on the right combination of α and β.

support. Past a certain threshold, however, the difference becomes negligible (Figure 4) and more remarkably, the qualitative dynamics are unchanged by any change in the microenvironment. The same effect is evident in the other two parameters, the ratio of symmetric vs asymmetric division of SCs and the proliferative potential of progenitor cells. Regardless of the vascularisation, disruption of homeostasis only occurs when the proliferative potential of progenitor cells (β) is below a maximum value of about 15. For values of symmetric division (α) above or below 0.3, the values for β in which this overgrowth occurs becomes even more restrictive with a range of approximately 10-15 allowing for maximum growth for $\alpha>0.3$ and a peak value of 5 for $\alpha<0.3$ (although with smaller total cell numbers).

Discussion:

In this paper we have presented a simple computational model of the HM of SC driven tissue growth. Our results show that there are distinct regions in parameter space (that directly correlate to the intrinsic SC phenotype space) that encode vastly different behaviour in the tissue (or tumour) arising from the SC in question. These parameters represent different SC phenotypes, and therefore do not represent any specific genetic mutation. In this way, we hope to generalise the intrinsic alterations which a SC could undergo much in the same way that the 'hallmarks of cancer' have generalised non-SC specific alterations [23] - with the end goal being

the identification of treatment strategies to target these phenotypes to slow or stop the progression of a SC driven cancer.

Because of the difficulties in understanding TIC specific traits *in vivo*, the biological data to support these conclusions remains sparse. There have been some carefully undertaken *in vitro* experiments on single TICs in glioblastoma, a highly invasive and malignant brain tumour, which suggest that TIC specific division behaviour (symmetric division rate) is variable and changes based on environmental cues [8]. Further work has shown that the other microenvironmental cues, such as acidity [11] and hypoxia [24-30] can also alter the prevalence of the stem phenotype by utilising functional markers of stemness, but the mechanism for this increase is as of yet imperfectly understood.

Of note, our simulations do not show a significant TIC population dependence on hypoxia (Fig. 3), or a change in stem composition (data not shown), suggesting a flaw in the model. To rectify this, future iterations of this model should include direct feedback onto the cellular parameters from the microenvironment. This dependence is something which could also be parameterised by specific *in vitro* experiments designed to quantify this effect, rather than just elucidating its existence. Other future developments of this model should take into consideration the emerging body of work suggesting that the proportion of TICs within a tumour is directly affected by therapy. There is now evidence in several cancers to suggest that radiation increases the size of the TIC pool. Specifically, in breast cancer, it has been shown that radiation therapy induces non-stem cancer cells to de-differentiate into TICs [31] - a behaviour not yet considered in any spatial theoretical models, but one that is gaining more and more attention [32] and which has been modelled with a deterministic ordinary differential equation system for a well-mixed population of cells [33]. Further, radiation has been shown to increase the TIC pool in glioblastoma [34], which has often been attributed to radiation resistance [13] and a new study by Gao et al. [35] has shown *in silico* and *in vitro* that radiation can effect the symmetric to asymmetric division ratio (our intrinsic parameter α), yielding further clues about the mechanism of this TIC pool expansion.

We, as well as others, find that the HM of tissue growth does not completely capture all the necessary dynamics that characterise cancer growth - but there is still a great deal of understanding to be gained from studying this formalism. To this end, we have performed a study of the factors related to TICs driving this dynamic and have identified several key factors which promote increased growth of the resultant tumour. In the same way that Weinberg et al. have simplified the myriad (epi)genetic alterations which a tumour can undergo into the 'hallmarks of cancer' we seek to distill the traits of TICs in the same way. Specifically, we have found that the number of allowed divisions of TACs exhibits bounds outside of which tumour growth is unsustainable. This finding has been corroborated independently by recent work from Enderling and colleagues[36]. Further, there is a specific balance of symmetric to asymmetric division which keeps tumours from overgrowing; almost acting as a phenotypic 'tumour suppressor'. Indeed, changes in this ratio have been recently hypothesized to underlie the increasing stem pool in glioblastoma after irradiation [35].

Conclusions

We have presented a minimal spatial Hybrid Cellular Automaton model of the HM of a stem cell-driven tissue in which we have explored generalised SC phenotypic traits and have identified several key behaviours which influence the overall tissue behaviour. While our model does capture a number of salient phenotypic characteristics of TICs that seem to be conserved, it fails to capture the recently observed changes in stem fraction secondary to microenvironmental perturbations. This is an indication that any computational model of a stem-hierarchical tissue,

or tumour, built from this point on must not only include the physical microenvironment, but also feedback from the microenvironment onto the specific cellular parameters encoded in the HM.

Therefore, this endeavour has identified the crucial point that the microenvironment must effect the behaviour of the cells within the HM, and also several conserved phenotypic 'hallmarks', which could be the result of any number of (epi)genetic alterations or microenvironmental perturbations. By focussing on phenotype instead of genotype, and identifying the key points of the HM of stem-cell driven tumour growth, we have provided a beginning to identification of the therapeutic targets to a more tractable set as compared to the panoply of possible mutations encoding similar traits. Only with this sort of distillation of the biological complexity inherent to cancer initiation (and indeed progression) can we hope to make progress against this disease.

Acknowledgements

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