Biphasic modulation of cancer stem cell-driven solid tumour dynamics in response to reactivated replicative senescence

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Abstract

Objectives: Cell senescence is a physiological programme of irreversible mitotic arrest that is triggered after a variety of intracellular and extracellular events. Its purpose is to protect tissue integrity by disabling mitosis in stressed or damaged cells. The senescence program serves as a tumour suppressor, and cancer cells are believed to bypass senescence to advance to malignancy. Recent studies have shown that senescence can be reactivated in cancer cells through a number of external perturbations, including oncogene activation, tumour suppressor gene withdrawal and irradiation.

Materials and methods: We have developed an agent-based model of solid tumour growth whose input population composition is based on the cancer stem-cell hypothesis. It is used to show how cancer stem cells can drive tumour progression, while non-stem cancer cells (CCs) interfere with this by impeding cancer stem-cell dynamics.

Results: Here we show that intratumoural competition between the two cell types may arise to modulate tumour progression and ultimately cancer presentation risk. Model simulations reveal that reactivation of the replicative senescence programme in CCs initially increases total tumour burden, as attrition from cell death is partially averted, but evolves to provide tumour control in the long-term through increasing constraints on stem-cell compartment kinetics.

Conclusions: Reactivation of replicative senescence can prolong CC competition with cancer stem cells, thereby ultimately inhibiting malignant progression regardless of tumour size.

Introduction

In 1961, Hayflick and Moorhead reported that extensively cultured human primary cells cease to proliferate after a finite number of divisions (1) yet remain viable and metabolically active (2,3). Experiments with transfected telomerase, an enzyme that rebuilds telomeric DNA (4), revealed that proliferation exhaustion is a consequence of telomere erosion (5,6). This irreversible cytostasis is termed replicative senescence. Several other factors can lead to different forms of irreversible mitotic arrest, such as DNA damage and oxidative stress [stress-induced senescence; (3)], oncogenic activation [oncogene-induced senescence; (7)], or loss of tumour suppressor genes [PTEN loss-induced cellular senescence; (8)]. Senescent cells display altered cell morphology, overexpress plasminogen activator protein 1 and present β -galactosidase (9,10). Initially, cellular senescence was believed to be a side effect of culturing cells *in vitro*, but recently senescent cells have also been detected *in vivo* in a variety of tissues in a number of different organs (11). The senescence program is believed to protect tissue integrity by disabling mitosis in stressed or damaged cells – thus probably acting as a tumour suppressor (12). During carcinogenesis, cells acquire traits that enable circumvention of replicative senescence – a hallmark of cancer (13–15). It has been suggested, however, that the cellular senescence pathways remain intact in tumours (16) and can be reactivated through cell autonomous programs (17). It has been reported that cellular senescence can also be triggered, and might actually be the predominant response to ionizing radiation and many currently used chemotherapeutic drugs (3,15,18). Cell population growth arrest in these

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cases is achieved and maintained, in part, by increased expression of specific cyclin-dependent kinase inhibitors, including p16Ink4a (19).

Replicative senescence might play an important role in tumour growth kinetics. If cell death is averted in favour of senescence, the number of cells inevitably increases. Senescent cells, however, do not actively contribute to population expansion, and might even hinder non-senescent cell proliferation through competition for common resources. At the same time, there is a growing body of evidence supporting the cancer stem-cell (CSC) hypothesis as a hierarchical model for tumour cell populations (20,21). To rigorously understand the action of senescence within this hierarchical population structure, a theoretical framework has been developed that is capable of tracking how these dynamics interact.

The CSC hypothesis itself offers one unifying explanation for the seemingly disparate observations that (i) locally recurring cancers are commonly more aggressive than the original primary tumour from which they arise (22,23), while (ii) indolent tumours are exceedingly common among asymptomatic adults (24–26). We propose that these phenomena may be reconciled by the prospect that a sensitive cancer cell subpopulation competes with a minor, but more resistant and aggressive cancer cell subpopulation to prevent its enrichment beyond a small fraction. In the context of the CSC hypothesis, the sensitive fraction would be identified with non-stem cancer cells (CCs), and the resistant fraction with CSCs (27–30). Unique to CSCs is the capacity for self-renewal and for tumour initiation (31,32). CCs, in contrast, although being fully transformed, have only a limited lifespan and proliferation capacity and hence are incapable of initiating, reinitiating and sustaining tumours (33). CSCs have been shown to be intrinsically less sensitive to conventional types of treatment (28,34) and thus be likely to drive the frequent, aggressive recurrences seen after apparently eradicated tumour treatment (35). Cells sensitive to radiation and chemotherapeutic cell killing might also be susceptible to agents that work by other means. It has been shown that cancer cell fate can be shifted towards senescence using irradiation or moderate doses of chemotherapy (36), Kras or c-Myc inactivation in oncogene-addicted tumours (7,17) or loss of tumour suppressor Pten (8). Here we present a quantitative model of CSC-driven tumour progression, and investigate how re-induction of replicative senescence in CCs modulates tumour growth kinetics in early tumour development as well as in tumours that are already advanced.

Materials and methods

We introduce a theoretical framework to investigate reactivation of senescence as a modulator of tumour pro-

gression. While a number of quantitative models have been proposed to study a variety of aspects of cellular senescence (37–43), the model presented here considers growth modulation of CSC-driven tumours. We use an agent-based model to simulate the dynamics and interactions of single cells and observe emergent tumour formation (33,44). Agent-based modelling is a computational methodology for simulating intercellular and cell–environment interactions that can reveal emerging properties of a dynamic system in time and space; it has been widely used in diverse areas of biomedical research (45,46) including cancer and tumour biology (47–54). In agent-based models, the system is represented as a collection of autonomous, decision-making agents, or cells, that have a set of intrinsic state variables and predefined instructions that determine how they behave and interact with each other and their local environment. Full details of cells and their interactions as the simulation progresses are visualizable, measurable and accessible to intervention (55). Stochastic simulations of the interaction of single cells and multiple cell populations with one another as well as with their immediate environment result in complex population dynamics such as cooperation or competition (56). The agent-based framework can capture the complex interactive consequences of these dynamics while allowing for distinction of the various participating cell populations of CSCs, CCs and senescent non-stem cancer cells (SCCs).

The model is based on asynchronous cellular automata in which all cell events are stochastically driven. A cell is an individual entity that occupies a single grid point of $100 \mu\text{m}^2$ on a two-dimensional or $1000 \mu\text{m}^3$ on a three-dimensional cubic lattice. All tumour subpopulations, CSCs, CCs and SCCs, are characterized by their proliferation potential. We have assumed that only CSCs and CCs are able to divide and produce daughter cells, while SCCs are in an irreversible post-mitotic state. According to the CSC hypothesis, CSCs are immortal and have infinite proliferative potential. At each division, CSCs produce either another CSC with probability $0 < P_s \leq 1$ (symmetric division) or a CC with probability $1 - P_s$ (asymmetric division). CCs that are direct offspring of a CSC are equipped with a limited proliferation capacity $\rho = \rho_{\text{max}}$ (Fig. 1a) that reduces with each cell division, a quantitative rendering of telomere shortening (9). At $\rho = 0$, CCs either die or become senescent. We assume that post-mitotic fate is decided randomly with probability $0 \leq \sigma \leq 1$, where $\sigma = 0$ simulates cell kinetics without senescence (that is, all cells will die after exhaustion of proliferation capacity), and $\sigma = 1$ simulates exclusive induction of senescence after exhaustion of proliferation capacity. Dead cells are instantaneously removed from the simulation, whereas

Figure 1. (a) Proliferation hierarchy of cancer stem cells (CSCs), non-stem cancer cells (CCs) and senescent cancer cells (SCCs). (b, c) Tumour cells populate the computational lattice by cell migration and cell proliferation. A cell can randomly migrate to, or place a daughter cell in, one of the eight (b) or twenty-six (c) adjacent lattice points subject to availability. A cell becomes quiescent if all adjacent lattice points are occupied.

senescent cells remain components of the tumour system. Due to continued metabolic activity and absence of cell division, senescent cells commonly increase in size but have reduced motility (57–59). While CSCs and CCs are equipped with fixed migration rate μ , we have investigated senescent cells with either an equivalent migration rate $\mu_s = \mu$ or lower motility assumed to be $\mu_s = 0.25 \mu$, which represents 75% reduction in cell motility. Cell proliferation and cell migration are dependent on the intracellular state as well as the local environment. We assume that cells need adjacent space for migration and proliferation, and cells that are completely surrounded by other cells (eight or twenty-six on a two- or three-dimensional lattice respectively) become quiescent (Fig. 1b,c) until neighbouring space opens up *via* cell migration or spontaneous cell death. In unsaturated environments, cells proliferate and migrate into vacant adjacent space at random.

To simulate tumour progression, we have initialized tumour growth with one CSC in the centre of the computational lattice. We chose lattice sizes of 600×600 (representing a 36 mm^2 domain) in two dimensions and $500 \times 500 \times 500$ (representing a 125 mm^3 domain) in three dimensions respectively. We assume that cells divide on average once per day and set cell cycle length to be 24 h (60). Furthermore, in line with previous models, we assume cell migration to be in the order of $\mu = 150 \text{ } \mu\text{m/day}$ (33), and thus migration into adjacent lattice points (10–14 μm away) can occur within 96 min. Therefore, we advance time at discrete time intervals $\Delta t = 1/24 \text{ day}$ (60 min). At each simulation

time step, cells are considered in random order and behaviour of each is updated. Cell proliferation and migration are temporally mutually exclusive events, and spontaneous cell death, as for example, due to metabolic catastrophe (61), occurs during proliferation attempts. We set spontaneous cell death rate to $\alpha = 0.01$ to allow for natural, attrition-dependent tumour progression. We assume migration probabilities $P_m = \mu \times \Delta t$ for CCs and CSCs and $P_m = \mu_s \times \Delta t$ for SCCs, where parameters μ and μ_s ($\mu_s \leq \mu$) denote motility of non-senescent and senescent cancer cells (SCCs), respectively. To reflect the low frequency of CSCs in solid tumours reported in the literature (29), we set probability of symmetric CSC division (which yields two CSCs) to $P_s = 0.01$. With probability $1 - P_s$, CSC division yields a CSC and a CC. Motivation for and variation of mentioned parameters α , μ and P_s and their impact on tumour progression have been discussed in detail elsewhere (33,44,62,63). Figure 2 summarizes the simulation procedure for the presented model. Due to the model's stochastic nature, we performed 100 independent simulations for each discussed parameter set and report average values and standard deviations.

Results

We first simulated CSC-driven tumour growth without replicative senescence. Then we investigated how early replicative senescence modulates intrinsic tumour progression, and studied effects of reactivated replicative senescence in established tumours of different sizes.

Figure 2. Schematic representation of the simulation process.

Cancer stem cell-driven self-metastatic tumour progression

We simulated tumour progression initiated by one CSC for $t = 1460$ days (4 years). We compared tumour progression as a function of the CC proliferation capacity $\rho_{\max}$ (c.f. Fig. 1a) – low proliferation capacity $\rho_{\max} = 10$ and larger proliferation capacity $\rho_{\max} = 20$ divisions. As previously discussed (33,44), with low probability of symmetric CSC division $P_s = 0.01$, tumour growth is limited by tumour mass (64). The founding CSC quickly produces CCs that go on and produce exclusively more CCs. As symmetric CSC division is an infrequent event, the CSC rapidly becomes engulfed in the CC population. The interior of the population enters quiescence due to spatial constraints (c.f. Fig. 1b,c) and proliferation is limited to the outer rim of the growing population. Proliferation capacities of proliferating CCs near the outer rim continuously reduce and peripheral cell death sets in after proliferation capacity is exhausted ($\rho = 0$; $\sigma = 0$). Previously quiescent CCs become active again and follow the kinetics of their predecessors. Tumour population size oscillates until the CSC becomes liberated and recommences proliferation, subject to (a)symmetric division. If symmetric division produces a new CSC, the two CSCs can opportunistically spatially separate and form independent clusters of self-limited size in the vicinity of each other (Fig. 3). This process repeats with increasing frequency as a continuously increasing number of CSCs seed new CSCs

and new tumour clusters nearby – a process we and others have termed self-metastatic tumour progression (44,63,65).

Figure 3 shows that tumours with a larger CC proliferation capacity $\rho_{\max} = 20$ have significantly slower tumour growth. Inhibition of the founding CSC by its early progeny CCs and their subsequent offspring is so profound that in the simulated time interval of 4 years, no tumour progression beyond the initial self-limited population is observed. A second CSC forms, but inhibition by progeny of both CSCs limits spatial separation and self-metastatic progression (44). Faster advance of the population with $\rho_{\max} = 10$ occurs despite the fact that a CC with $\rho_{\max} = 10$ will over time generate a population of $2^{10} = 1024$ cells, whereas the population arising from a CC with $\rho_{\max} = 20$ will consist of $2^{20} = 1\,048\,576$ cells – more than three orders of magnitude (>1000 times) more cells. In the next sections, we report our investigation of impact of replicative senescence on slowly progressing ($\rho_{\max} = 20$) and rapidly progressing ($\rho_{\max} = 10$) CSC-driven self-metastatic tumours.

Replicative senescence and early tumour progression modulation

We then introduced replicative senescence into the model. We first assumed $\sigma = 1$, total deferment of cell death in favour of senescence. A CC that exhausts its replicative potential, $\rho = 0$, will become senescent (SCC) and avoid apoptosis. To account for reduced

Figure 3. Self-metastatic tumour growth. Cancer stem cells (CSC, yellow) form small tumour clusters and seed independent clusters in their vicinity. Efficiency of self-metastatic seeding depends on inhibition by non-stem cancer cells. Larger proliferation potentials ($\rho_{\max} = 20$) can inhibit self-metastatic seeding and induce prolonged phases of dormancy.

motility of SCCs (58,59) we compared migration rates of μ_s for the cases $\mu_s = \{\mu, 0.25\mu\}$. Effect of SCC presence on tumour progression is biphasic. Initially, introduction of replicative senescence yields larger tumours with larger total number of cells compared to self-metastatic tumour progression, as cell death is averted. As one would expect (44), lower SCC migration rate $\mu_s = 0.25\mu$ results in smaller tumours as competition with and restrictions on mitotic populations are increased. Interestingly, tumour morphology changes from a self-metastatic phenotype to compact circular tumours with a diffuse rim (Fig. 4) that is characteristic of cell diffusion-driven tumours (66,67). As SCCs cease to proliferate and thus no longer increase cell number, and further refrain from vacating the space they occupy and therefore inhibit CC and CSC proliferation, tumour

progression is severely impaired in the long term. Consequently, a reverse trend in tumour numbers can be observed and tumours with SCCs become smaller with significantly reduced growth rates at later times compared to self-metastatic tumours without senescence (Fig. 4). Rapidly growing self-metastatic tumours (i.e. $\rho_{\max} = 10$) contain an order of magnitude more cells and two orders of magnitude more CSCs than SCC-harboured tumours, in which only 1.05% (for $\mu_s = \mu$) and 1.19% ($\mu_s = 0.25\mu$) are non-senescent with remaining proliferation capacity $\rho > 0$.

The effect of early replicative senescence in slowly progressing self-metastatic tumours is remarkably different. If self-metastatically progressing tumours have a long initial self-limited, dormant phase (i.e. $\rho_{\max} = 20$), introduction of senescence increases total tumour cell

Figure 4. Comparison of tumour growth dynamics over time in two-dimensional lattice in cases of presence (light and dark green) or absence (Control, light yellow) of senescence, and for different replicative potential $\rho_{\max} = 10, 20$ and two migration potentials of senescent cells, $\mu_s = \mu$ or 0.25μ . (a) Typical tumour morphologies at $t = 1460$ days. s : CSC count; n : CC+SCC count. (b–d) Average statistics after 1460 days (bar plots) and (e) tumour evolution over time for $\rho_{\max} = 10$.

count as cell death is averted, but has little effect on CSC numbers or non-senescent CC counts (Fig. 4). To ensure that observed dynamics are not artefactual of the two-dimensional simulation setup, we extended the model into three dimensions (Fig. 5). Induction of replicative senescence in CC was confirmed biphasic as discussed above with initial increase in tumour cell count followed by reduction of tumour growth rate and inhibited tumour progression compared to self-metastatic tumour progression. Regardless of SCC migration rate μ_s , non-senescent cells (CSCs + CCs) make up less than 1% of the total population. Biphasic modulation of CSC-driven solid tumour growth can be observed even if only a very small fraction of CC avert replicative cell death in favour of senescence. Figure 6 shows the case for $\sigma = 0.02$, that is, 2% of CCs become senescent when their replicative potential is exhausted.

Reactivation of replicative senescence in established tumours

We simulated rapid self-metastatic tumour progression ($\rho_{\max} = 10$) and introduced exclusive replicative senescence with $\sigma = 1$, after tumours had reached sizes of 10 000, 50 000 or 100 000 cells. Induction of replicative senescence initially increased total population size as cell death was averted, but, in the long term, impeded tumour progression compared to unperturbed self-metastatic growth (Fig. 7). Interestingly, dynamics of the tumour response to reactivated replicative senescence was independent of tumour size. For all studied tumour sizes, evolution of total cell count after reactivation of senescence exhibited the biphasic initial increase and

Figure 6. Analysis of senescence induction at very low rate $\sigma = 0.02$ (i.e. 2% of cancer cell enter senescence instead of replicative cell death). (a) Total cell count. (b) Cancer stem-cell count. (c) Cancer stem-cell fraction. (d) Pre-senescent fraction.

later reduced growth rate, and differences in times until intersections of senescent tumour growth trajectories with untreated control were insignificant (see Table 1). Number of CSCs and thus total number of non-senescent cells was significantly lower regardless of tumour size (Fig. 7).

Discussion

Transformed cells ‘learn’ to bypass physiological programs of irreversible growth arrest as part of the

Figure 5. Comparison of tumour growth dynamics over time in three-dimensional lattice in cases of presence (light and dark green) or absence (Control, light yellow) of senescence for $P_s = 0.01$, $\rho_{\max} = 15$, $\mu = 10$ and $\mu_s = \mu$ or 0.25μ . (a) Examples of tumour morphologies after 530 days. (b) Evolution of tumours over time.

Figure 7. Effect of reactivation of the senescence program in non-stem cancer cells. (a) Examples of tumour morphologies after 100, 200 and 800 days. s: CSC, n: CC+SCC. (b) Evolution of tumour populations of different initial sizes.

Table 1. Average time until intersection of the senescent tumour growth trajectories with non-senescent control for different initial population sizes and senescent cells' migrations potentials

Tumour size at senescence reactivation	$\mu_s = \mu$	$\mu_s = 0.25 \mu$
10 000 cells	646 days	343 days
50 000 cells	599 days	333 days
100 000 cells	607 days	337 days

carcinogenic cascade. Pathways underlying cellular senescence, however, have been shown to remain intact in tumours and thus are reactivatable and potentially exploitable for therapeutic intervention. Here, we have presented a theoretical model of CSC-driven solid tumour progression and explored how tumour dynamics

are modulated if replicative senescence is reactivated. We have described solid tumour growth as self-metastatic expansion resulting from competition for space between CSCs and their non-stem progeny. Large CC populations inhibit CSC kinetics required for tumour growth. In this model, replicative senescence works to impede tumour progression – independent of tumour size – by further increasing pressure on the CSC population that is inevitably forced to remain in the core of the population. Initial response to senescence activation, however, is an undesirable rapid increase in cell number, and thus tumour burden, as cell death is averted.

Introduction of irreversible growth arrest in cancer cells utilizing a built-in, but currently under-appreciated, program might, nevertheless, present an attractive

therapeutic target. Pro-senescence treatments may have been prematurely dismissed for a number of reasons. First, senescent cells may alter the tissue microenvironment by expressing growth factors, degradative enzymes and inflammatory cytokines (68), which may induce proliferation, dedifferentiation and invasion by premalignant cells (69). Secondly, growth-promoting signalling of residual populations of cancer cells, albeit senescent, may still be of concern (15), and only recently have studies emerged reporting that senescent cells may be cleared by the immune system (70). In contrast to conventional cancer treatments with rapid gross tumour reduction and eradication intent, pro-senescent treatment might initially increase number of cancer cells as cell death is averted. In terms of later response, however, reactivated senescence might avoid eradicating most tumour cells altogether, instead causing subpopulations to ultimately compete so as to push the tumour as a whole into a self-limiting state. The result would be to therapeutically maintain the tumour in a dormant state as a chronic asymptomatic condition as opposed to a frank disease. In conclusion, response of a tumour population to potential therapeutic induction of senescence, with its short-term burst but longer term suppression, may comprise a welcome dynamic reversal *vis-à-vis* conventional types of treatment where an initial reduction is seen in tumour volume – often many orders of magnitude below level of clinical detection – followed by accelerated repopulation beyond initial tumour burden (23,35) that often proves fatal. These observations motivate investigations into combining conventional treatments that reduce tumour volume with subsequent pro-senescence therapy to maintain the reduced tumour in a controlled, dormant-like state.

In the presented model, senescent cells were shown to act to inhibit kinetics of pre-senescent cells. Clearance of senescent cells dampen the observed modulation towards tumour control, which suggests that failure to remove these post-mitotic cells may not be a major concern. The model, however, needs to be extended to account complex crosstalk of senescent cells with pre-senescent populations, adjacent non-transformed tissue cells and the host immune system (10,69,71) – all of which are expected to alter intrinsic tumour kinetics directly as well as indirectly by modulation of the senescent cell population and the supporting host.

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References

- Hayflick L, Moorhead PS (1961) The serial cultivation of human diploid cell strains. *Exp. Cell Res.* **25**, 585–621.
- Campisi J, d'Adda di Fagagna F (2007) Cellular senescence: when bad things happen to good cells. *Nat. Rev. Mol. Cell Biol.* **8**, 729–740.
- Kondoh H, Maruyama T, Lleonart ME (2008) Senescence as a target for cancer therapy. *Open Pathol.* **2**, 57–62.
- Greider CW, Blackburn EH (1985) Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. *Cell* **43**, 405–413.
- Blackburn EH, Gall JG (1978) A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in Tetrahymena. *J. Mol. Biol.* **120**, 33–53.
- Serrano M, Blasco MA (2001) Putting the stress on senescence. *Curr. Opin. Cell Biol.* **13**, 748–753.
- Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW (1997) Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* **88**, 593–602.
- Chen Z, Trotman LC, Shaffer D, Lin H-K, Dotan ZA, Niki M *et al.* (2005) Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. *Nat. Cell Biol.* **436**, 725–730.
- Campisi J (1997) The biology of replicative senescence. *Eur. J. Cancer* **33**, 703–709.
- Rodier F, Campisi J (2011) Four faces of cellular senescence. *J. Cell Biol.* **192**, 547–556.
- Collado M, Gil J, Efeyan A, Guerra C, Schuhmacher AJ, Barradas M *et al.* (2005) Tumour biology: senescence in premalignant tumours. *Nature* **436**, 642.
- Lynch MD (2006) How does cellular senescence prevent cancer? *DNA Cell Biol.* **25**, 69–78.
- Hanahan D, Weinberg RA (2000) The hallmarks of cancer. *Cell* **100**, 57–70.
- Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* **144**, 646–674.
- Lleonart ME, Artero-Castro A, Kondoh H (2009) Senescence induction: a possible cancer therapy. *Mol. Cancer* **8**, 3.
- Schmitt CA, Fridman JS, Yang M, Lee S, Baranov E, Hoffman RM *et al.* (2002) A senescence program controlled by p53 and p16INK4a contributes to the outcome of cancer therapy. *Cell* **109**, 335–346.
- Wu C-H, van Riggelen J, Yetil A, Fan AC, Bachireddy P, Felsher DW (2007) Cellular senescence is an important mechanism of tumour regression upon c-Myc inactivation. *Proc. Natl. Acad. Sci. USA* **104**, 13028–13033.
- Nardella C, Clohessy JG, Alimonti A, Pandolfi PP (2011) Pro-senescence therapy for cancer treatment. *Nat. Rev. Cancer* **11**, 503–511.
- Ewald JA, Desotelle JA, Wilding G, Jarrard DF (2010) Therapy-Induced Senescence in Cancer. *J. Natl. Cancer Inst.* **102**, 1536–1546.

- 20 Bonnet D, Dick JE (1997) Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat. Med.* **3**, 730–737.
- 21 Dick JE (2008) Stem cell concepts renew cancer research. *Blood* **112**, 4793–4807.
- 22 Roberts SA, Hendry JH (1993) The delay before onset of accelerated tumour cell repopulation during radiotherapy: a direct maximum-likelihood analysis of a collection of worldwide tumour-control data. *Radiother. Oncol.* **29**, 69–74.
- 23 Schmidt-Ullrich RK, Contessa JN, Dent P, Mikkelsen RB, Valerie K, Reardon DB *et al.* (1999) Molecular mechanisms of radiation-induced accelerated repopulation. *Radiat. Oncol. Investig.* **7**, 321–330.
- 24 Black WC, Welch HG (1993) Advances in diagnostic imaging and overestimations of disease prevalence and the benefits of therapy. *N. Engl. J. Med.* **328**, 1237–1243.
- 25 Folkman J, Kalluri R (2004) Cancer without disease. *Nature* **427**, 787.
- 26 Welch HG, Black WC (2010) Overdiagnosis in Cancer. *J. Natl. Cancer Inst.* **102**, 605–613.
- 27 Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J *et al.* (2003) Identification of a cancer stem cell in human brain tumours. *Cancer Res.* **63**, 5821–5828.
- 28 Baumann M, Krause M, Hill R (2008) Exploring the role of cancer stem cells in radioresistance. *Nat. Rev. Cancer* **8**, 545–554.
- 29 Visvader JE, Lindeman GJ (2008) Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nat. Rev. Cancer* **8**, 755–768.
- 30 Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF (2003) Prospective identification of tumorigenic breast cancer cells. *Proc. Natl. Acad. Sci. USA* **100**, 3983–3988.
- 31 Morrison SJ, Kimble J (2006) Asymmetric and symmetric stem-cell divisions in development and cancer. *Nature* **441**, 1068–1074.
- 32 Caussinus E, Hirth F (2007) Asymmetric stem cell division in development and cancer. *Prog. Mol. Subcell. Biol.* **45**, 205–225.
- 33 Enderling H, Anderson ARA, Chaplain MAJ, Beheshti A, Hlatky L, Hahnfeldt P (2009) Paradoxical dependencies of tumour dormancy and progression on basic cell kinetics. *Cancer Res.* **69**, 8814–8821.
- 34 Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB *et al.* (2006) Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* **444**, 756–760.
- 35 Enderling H, Chaplain MAJ, Hahnfeldt P (2010) Quantitative modeling of tumour dynamics and radiotherapy. *Acta. Biotheor.* **58**, 341–353.
- 36 Roninson IB (2003) Tumour cell senescence in cancer treatment. *Cancer Res.* **63**, 2705–2715.
- 37 Buijs JOD, van den Bosch PPJ, Musters MWJM, van Riel NAW (2004) Mathematical modeling confirms the length-dependency of telomere shortening. *Mech. Ageing Dev.* **125**, 437–444.
- 38 Karpins TV, Foy BD (2004) Model of the developing tumorigenic phenotype in mammalian cells and the roles of sustained stress and replicative senescence. *J. Theor. Biol.* **227**, 253–264.
- 39 Wang Y, Aguda BD, Friedman A (2007) A continuum mathematical model of endothelial layer maintenance and senescence. *Theor. Biol. Med. Model.* **4**, 30.
- 40 Wodarz D (2007) Effect of stem cell turnover rates on protection against cancer and aging. *J. Theor. Biol.* **245**, 449–458.
- 41 Mitteldorf J, Pepper J (2009) Senescence as an adaptation to limit the spread of disease. *J. Theor. Biol.* **260**, 186–195.
- 42 Marciniak-Czochra A, Stiehl T, Wagner W (2009) Modeling of replicative senescence in hematopoietic development. *Aging (Albany NY)* **1**, 723–732.
- 43 La Porta CAM, Zapperi S, Sethna JP (2012) Senescent cells in growing tumours: population dynamics and cancer stem cells. *PLoS Comput. Biol.* **8**, e1002316.
- 44 Enderling H, Hlatky L, Hahnfeldt P (2009) Migration rules: tumours are conglomerates of self-metastases. *Br. J. Cancer* **100**, 1917–1925.
- 45 Deutsch A, Dormann S (2005) Cellular Automaton Modeling of Biological Pattern Formation: Characterization, Applications, and Analysis. Boston, MA: Birkhäuser.
- 46 Anderson ARA, Chaplain MAJ, Rejniak KA (2009) Single-Cell-Based Models in Biology and Medicine. Basel, Switzerland: Birkhäuser.
- 47 Rejniak KA (2007) An immersed boundary framework for modeling the growth of individual cells: an application to the early tumour development. *J. Theor. Biol.* **247**, 186–204.
- 48 Alarcón T, Byrne HM, Maini PK (2003) A cellular automaton model for tumour growth in inhomogeneous environment. *J. Theor. Biol.* **225**, 257–274.
- 49 Gerlee P, Anderson ARA (2007) An evolutionary hybrid cellular automaton model of solid tumour growth. *J. Theor. Biol.* **246**, 583–603.
- 50 Wang Z, Zhang L, Sagotsky J, Deisboeck TS (2007) Simulating non-small cell lung cancer with a multiscale agent-based model. *Theor. Biol. Med. Model.* **4**, 50.
- 51 Basanta D, Simon M, Hatzikirou H, Deutsch A (2008) Evolutionary game theory elucidates the role of glycolysis in glioma progression and invasion. *Cell Prolif.* **41**, 980–987.
- 52 Enderling H, Alexander NR, Clark ES, Branch KM, Estrada L, Crooke C *et al.* (2008) Dependence of invadopodia function on collagen fiber spacing and cross-linking: computational modeling and experimental evidence. *Biophys. J.* **95**, 2203–2218.
- 53 Piotrowska MJ, Angus SD (2009) A quantitative cellular automaton model of in vitro multicellular spheroid tumour growth. *J. Theor. Biol.* **258**, 165–178.
- 54 Mukhopadhyay R, Costes SV, Bazarov AV, Hines WC, Barcellos-Hoff M, Yaswen P (2010) Promotion of variant human mammary epithelial cell outgrowth by ionizing radiation: an agent-based model supported by in vitro studies. *Breast Cancer Res.* **12**, R11.
- 55 Tang J, Enderling H, Becker-Weimann S, Pham C, Polyzos A, Chen C-Y *et al.* (2011) Phenotypic transition maps of 3D breast acini obtained by imaging-guided agent-based modeling. *Integr. Biol.* **3**, 408.
- 56 Power C (2009) A spatial agent-based model of n-person Prisoner's Dilemma cooperation in a socio-geographic community. *J. Artif. Soc. Soc. Sim.* **12**, 8.
- 57 Hayflick L (1965) The limited in vitro lifetime of human diploid cell strains. *Exp. Cell Res.* **37**, 614–636.
- 58 Xu D, Takeshita F, Hino Y, Fukunaga S, Kudo Y, Tamaki A *et al.* (2011) miR-22 represses cancer progression by inducing cellular senescence. *J. Cell Biol.* **193**, 409–424.
- 59 Korybalska K, Kawka E, Kusch A, Aregger F, Dragun D, Jorres A *et al.* (2013) Recovery of senescent endothelial cells from injury. *J. Gerontol. A Biol. Sci. Med. Sci.* **68**, 250–257.
- 60 Gao X, McDonald JT, Hlatky L, Enderling H (2013) Acute and fractionated irradiation differentially modulate glioma stem cell division kinetics. *Cancer Res.* **73**, 1481–1490.
- 61 Jin S, DiPaola RS, Mathew R, White E (2007) Metabolic catastrophe as a means to cancer cell death. *J. Cell Sci.* **120**, 379–383.
- 62 Enderling H, Park D, Hlatky L, Hahnfeldt P (2009) The importance of spatial distribution of stemness and proliferation state in determining tumour radioresponse. *Math. Model. Nat. Phenom.* **4**, 117–133.

- 63 Enderling H, Hlatky L, Hahnfeldt P (2010) Tumour morphological evolution: directed migration and gain and loss of the self-metastatic phenotype. *Biol. Direct* **5**, 23.
- 64 Prehn RT (1991) The inhibition of tumour growth by tumour mass. *Cancer Res.* **51**, 2–4.
- 65 Norton L (2005) Conceptual and practical implications of breast tissue geometry: toward a more effective, less toxic therapy. *Oncologist* **10**, 370–381.
- 66 Folkman J, Hochberg M (1973) Self-regulation of growth in three dimensions. *J. Exp. Med.* **138**, 745–753.
- 67 Roose T, Chapman SJ, Maini PK (2007) Mathematical models of avascular tumour growth. *SIAM Rev.* **49**, 179–208.
- 68 Jeyapalan JC, Sedivy JM (2008) Cellular senescence and organismal aging. *Mech. Ageing Dev.* **129**, 467–474.
- 69 Parrinello S (2005) Stromal-epithelial interactions in aging and cancer: senescent fibroblasts alter epithelial cell differentiation. *J. Cell Sci.* **118**, 485–496.
- 70 Xue W, Zender L, Miething C, Dickins RA, Hernando E, Krizhanovsky V *et al.* (2007) Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature* **445**, 656–660.
- 71 Davalos AR, Coppe J-P, Campisi J, Desprez P-Y (2010) Senescent cells as a source of inflammatory factors for tumour progression. *Cancer Metastasis Rev.* **29**, 273–283.