Heterogeneity for IGF-II production maintained by public goods dynamics in neuroendocrine pancreatic cancer

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Edited* by Peter T. Ellison, Harvard University, Cambridge, MA, and approved December 19, 2014 (received for review July 31, 2014)

The extensive intratumor heterogeneity revealed by sequencing cancer genomes is an essential determinant of tumor progression, diagnosis, and treatment. What maintains heterogeneity remains an open question because competition within a tumor leads to a strong selection for the fittest subclone. Cancer cells also cooperate by sharing molecules with paracrine effects, such as growth factors, and heterogeneity can be maintained if subclones depend on each other for survival. Without strict interdependence between subclones, however, nonproducer cells can free-ride on the growth factors produced by neighboring producer cells, a collective action problem known in game theory as the "tragedy of the commons," which has been observed in microbial cell populations. Here, we report that similar dynamics occur in cancer cell populations. Neuroendocrine pancreatic cancer (insulinoma) cells that do not produce insulin-like growth factor II (IGF-II) grow slowly in pure cultures but have a proliferation advantage in mixed cultures, where they can use the IGF-II provided by producer cells. We show that, as predicted by evolutionary game theory, producer cells do not go extinct because IGF-II acts as a nonlinear public good, creating negative frequency-dependent selection that leads to a stable coexistence of the two cell types. Intratumor cell heterogeneity can therefore be maintained even without strict interdependence between cell subclones. Reducing the amount of growth factors available within a tumor may lead to a reduction in growth followed by a new equilibrium, which may explain relapse in therapies that target growth factors.

game theory | tumor | evolution

Cancer is a process of clonal selection within the body on the time scale of an individual's lifetime (1–4): Tumor cells that reproduce more rapidly increase in frequency at the expense of neighboring healthy cells, even if this ■■■ is deleterious for the organism. For the same reason, cell subclones that have a proliferative advantage within the tumor are expected to drive other subclones to extinction. However, intratumor cell heterogeneity is commonly observed (5, 6). Despite the implications for cancer progression, diagnosis, and treatment (7–9), the mechanistic basis for this heterogeneity remains unclear (3, 9).

One possible answer comes from the observation that cancer cells not only compete for space and resources but also cooperate by sharing molecules with paracrine functions, such as growth factors. Because growth factors diffuse in the ECM, their effects are not limited to producer cells and can be considered a form of cooperation between cells (10). Heterogeneity can be maintained in case of strict interdependence between cell subclones because individual subclones are unable to proliferate autonomously but can complement each other's deficiency (10, 11), and hence coexist. An example of such a scenario has recently been reported in a mouse model of mammary cancer (12), where luminal cells secrete the oncogenic factor Wnt1 and basal cells carry a cancer-driving mutation in the Hras gene (11).

This kind of cooperation between subclones (11) is analogous to mutualism in ecology (10); yet, like mutualism in ecology, a strict interdependence is not always the case. More commonly, a mutation may simply impair the production of a growth factor, and thus create a nonproducer cell. This mutant "defector" cell will still be able to "free-ride" on the growth factors produced by its cooperative neighboring cells; hence, this subclone would be expected to have a higher fitness and take over the population. Although this kind of interaction has been studied extensively in bacteria (13), where it has implications for the evolution of resistance to antibiotics (14), and in yeast (15), it has received little attention in cancer research due, in part, to a lack of adequate experimental systems.

Here, we have analyzed the dynamics of cooperation and defection for the production of insulin-like growth factor II (IGF-II) in an experimental cancer system in vitro. IGF-II is an ideal growth factor to study cooperation and defection among cancer cells because it is up-regulated in many cancer types and has been shown to stimulate cell growth and to protect cells from apoptosis (16–19). We used β-tumor cell lines derived from insulinomas of Rip1Tag2 mice (20) [henceforth called "producer" cells (+/+)] and from the same transgenic mice carrying a homozygous deletion of the IGF-II gene (16) ["nonproducer" cells (−/−)] to investigate whether, in the absence of interdependence between subclones, cell heterogeneity can be maintained or nonproducer cells drive producer cells to extinction. As we shall see, cooperation and stable heterogeneity are observed, a result that we analyzed by resorting to evolutionary game theory (21).

Significance

Cancer cells compete for space and nutrients against healthy cells and other cancer cells but also cooperate by secreting growth factors. Clones that do not produce growth factors, however, have a proliferation advantage because they can use the factors produced by neighboring cells without the cost of producing them. Therefore, the cooperative production of growth factors by tumor cells should collapse. What maintains cooperation within the tumor? Here, we use evolutionary game theory to explain how heterogeneity can persist, and we use experiments with pancreatic cancer cells to test the predictions of the theory. Cancer is a process of clonal selection, and studying cancer cell populations using methods and concepts from evolutionary biology can reveal potential evolutionarily stable therapies.

Author contributions: M.A. designed research; M.A., D.A.F., and G.C. designed experiments; M.A. designed theory; M.A. and D.A.F. performed experiments; M.A. analyzed data; and M.A., D.A.F., and G.C. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

Freely available online through the PNAS open access option.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1414653112/-/DCSupplemental.

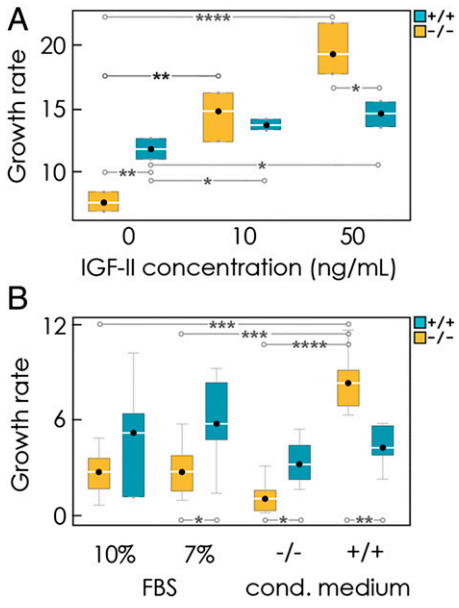

Fig. 1. IGF-II is a nonlinear public good. (*A*) Growth rates of producer (+/+) and nonproducer (–/–) cells in vitro (relative to day 1) at different concentrations of exogenous IGF-II in the growth medium. (*B*) Growth rates of +/+ and –/– cells in vitro (relative to the day with the minimum number of cells) with medium containing FBS (7% or 10%) or in conditioned (cond.) medium from –/– or +/+ cultures. Box plots show the median and the 25% and 75% quartiles (upper and lower fences, respectively). Asterisks show significant *P* values in a *t* test: **P* < 0.05; ***P* < 0.005; ****P* < 0.0005; *****P* < 0.00005.

Results

IGF-II Is an Intratumor Public Good. We first determined experimentally the growth rates of individual cultures. Pure cultures of –/– cells grew more slowly than pure +/+ cultures in the absence of exogenous IGF-II (Fig. 1*A*). This finding is not surprising, because IGF-II is known to enhance cell proliferation and survival. The growth rates of +/+ cultures were only marginally affected by exogenous IGF-II (Fig. 1*A*), suggesting that the IGF-II produced by the +/+ cells themselves is enough to sustain their proliferation. The growth rates of –/– cultures, on the other hand, increased at higher concentrations of IGF-II; more specifically, proliferation is a sigmoid function of IGF-II concentration (Fig. S1). Notably, at high concentrations of IGF-II, –/– cultures exhibited higher growth rates than pure +/+ cultures (Fig. 1*A*), indicating a cost for producing IGF-II.

If –/– cells can benefit from soluble IGF-II in the growth medium, they can arguably also benefit from the presence of IGF-II produced by +/+ cells. To verify whether this ■■■ is the case, we measured the growth rates of pure cultures in conditioned medium derived from –/– and +/+ pure cultures. Indeed, we observed that the growth rates of –/– cells improved when cultured in the presence of conditioned medium from +/+ cultures, whereas medium from –/– cultures had no significant effect (Fig. 1*B*). This finding suggests that a factor secreted by the +/+ cells is responsible for the increased growth of the –/– cells. Because the two cell lines differ in the production of IGF-II, this factor is arguably IGF-II itself.

The critical result was that –/– cells grew better than +/+ cells in medium conditioned by +/+ cells (Fig. 1*B*). This finding suggested that –/– cells would also outperform +/+ cells in mixed cultures if enough +/+ cells were cocultured. One could expect, therefore, that nonproducer cells would increase in frequency over time, because they are able to free-ride on the IGF-II produced (at a cost) by their +/+ cooperative neighbors. Similar observations have been made in bacteria (13, 14) and in yeast (15), a problem that is generally referred to as the "tragedy of the commons" in game theory (22): Because of the individual incentive to free-ride on other group member's contributions, a nonproducer has a fitness advantage that will eventually lead to the demise of the population because of the lack of public good. We experimentally tested this hypothesis by observing how mixed cultures of +/+ and –/– cells change over time in vitro.

In the presence of 10% FBS (the concentration generally used for maintaining β-tumor cells in culture), we observed a rapid decline in the frequency of +/+ cells in mixed populations. At lower serum concentrations, however, we observed the opposite: The +/+ genotype increased in frequency. At intermediate levels of serum, on the other hand, there was no clear winner: The two cell types coexisted (Fig. 2). Only when starting the culture with low initial frequencies of +/+ cells did they go extinct. The coexistence of –/– and +/+ cells is of particular interest for our understanding of intratumor cell heterogeneity. What maintains a mixed population? Why does this heterogeneity disappear at low and high levels of serum?

Nonlinear Benefits Maintain Heterogeneity. To decipher the growth dynamics observed in the coculture experiments, we resorted to evolutionary game theory (21). A +/+ cell pays a cost for producing the growth factor, which a –/– cell does not pay, yet the +/+ cell has a higher benefit because of the extra growth factor produced by itself. When the extra benefit offsets the cost, +/+ cells are predicted to increase in frequency, whereas they should decline in frequency when the cost is higher than the extra benefit (Fig. 3*A*) If this negative frequency-dependent benefit is nonlinear (because of synergistic effects and diminishing returns), clonal selection will lead to an increase of –/– cells when there are too many or too few +/+ cells, but not at intermediate frequencies. In other words, because IGF-II acts as a nonlinear public good (23), clonal selection can lead to a stable coexistence of +/+ and –/– cells if the cost of producing IGF-II is not too high (Fig. 3*B*).

If this interpretation is correct, we expect to observe that +/+ cells will go extinct in mixed populations when the cost/benefit ratio of producing IGF-II is too high. In contrast, at lower cost/benefit ratios, we should observe coexistence of the two cell types. Although the cost of IGF-II production is constant, we can change the benefit provided by endogenous IGF-II by varying the amount of serum in the medium: Because serum contains additional nutrients and growth factors (24) (including IGF-II), more serum reduces the relative benefit of the IGF-II produced by the +/+ cells, and thus increases its cost/benefit ratio. Likewise, reducing the concentration of serum in the medium increases the benefit of endogenously produced IGF-II, thus reducing its cost/benefit ratio. The bistability predicted by the theory (the existence of an internal unstable equilibrium, Fig. 3 *A* and *B*) would also explain why, given the same amount of FBS, +/+ cells sometimes went extinct when starting from low frequencies (Fig. 2).

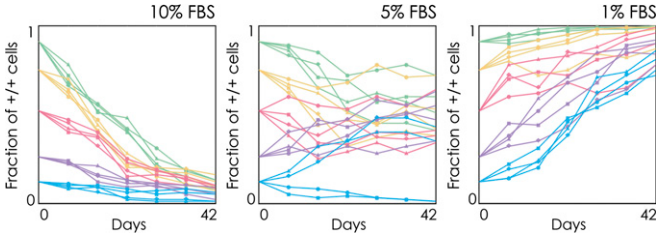

Fig. 2. Long-term dynamics of IGF-II production in vitro. Observed changes in the frequency of IGF-II–producing cells (+/+) in mixed populations of +/+ cells and –/– cells seeded at varying ratios and under different concentration of FBS in the culture medium; the cost/benefit ratio of IGF-II increases with the amount of FBS.

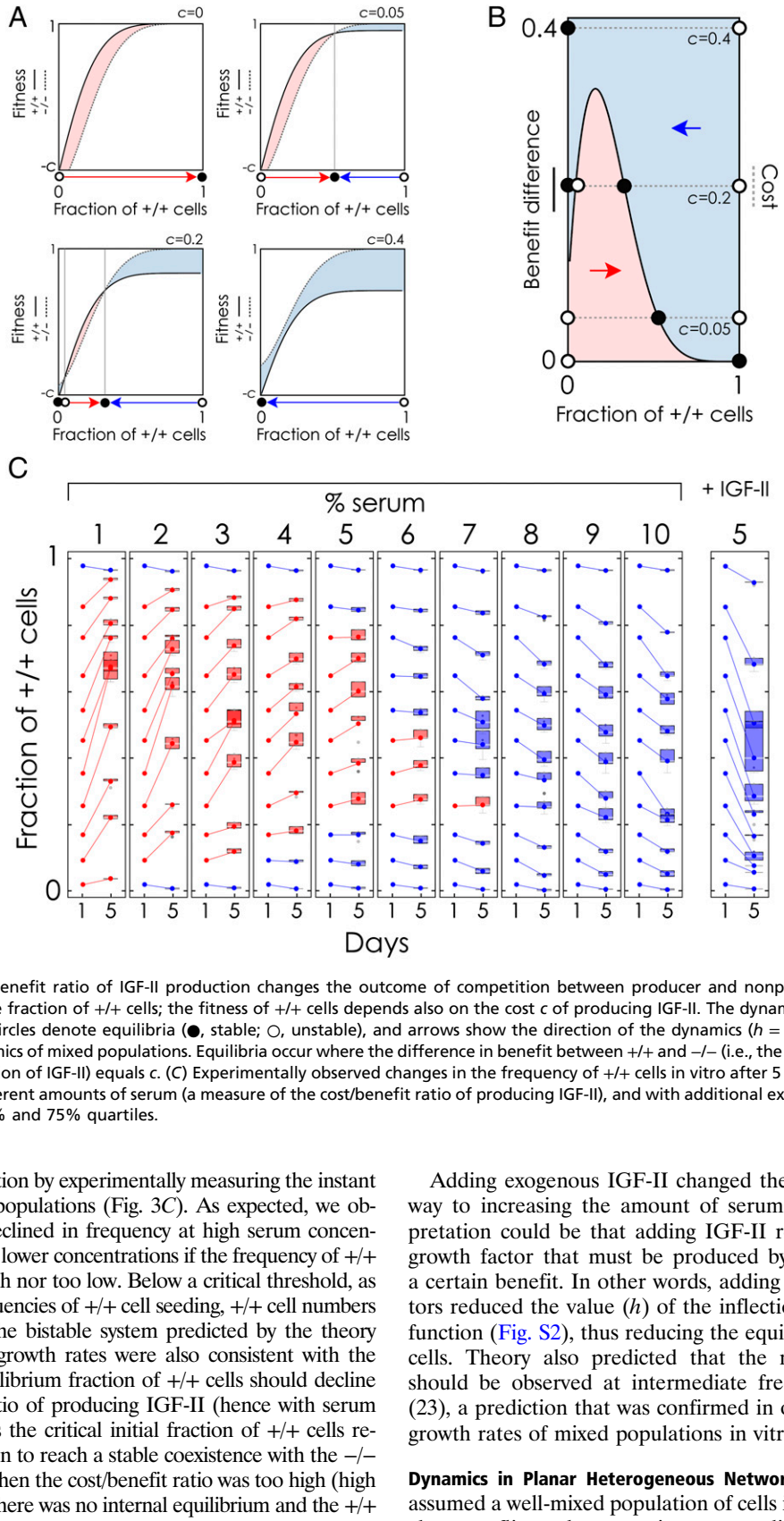

Fig. 3. Varying the cost/benefit ratio of IGF-II production changes the outcome of competition between producer and nonproducer cells. (*A*) Fitness is a nonlinear function of the fraction of +/+ cells; the fitness of +/+ cells depends also on the cost *c* of producing IGF-II. The dynamics depend on the relative fitness of the two types. Circles denote equilibria (●, stable; ○, unstable), and arrows show the direction of the dynamics ($h = 0.2$, $s = 10$, $n = 30$). (*B*) Alternative view of the dynamics of mixed populations. Equilibria occur where the difference in benefit between +/+ and −/− (i.e., the additional benefit for a +/+ cell due to its own production of IGF-II) equals *c*. (*C*) Experimentally observed changes in the frequency of +/+ cells in vitro after 5 d of coculture for different initial frequencies and different amounts of serum (a measure of the cost/benefit ratio of producing IGF-II), and with additional exogenous IGF-II (100 ng/mL). Error bars indicate the 25% and 75% quartiles.

We tested this prediction by experimentally measuring the instant growth rates of mixed populations (Fig. 3*C*). As expected, we observed that +/+ cells declined in frequency at high serum concentrations but increased at lower concentrations if the frequency of +/+ cells was neither too high nor too low. Below a critical threshold, as well as at very high frequencies of +/+ cell seeding, +/+ cell numbers decreased, leading to the bistable system predicted by the theory (Fig. 3*B*). The instant growth rates were also consistent with the prediction that the equilibrium fraction of +/+ cells should decline with the cost/benefit ratio of producing IGF-II (hence with serum concentration), whereas the critical initial fraction of +/+ cells required for the population to reach a stable coexistence with the −/− cells should increase. When the cost/benefit ratio was too high (high serum concentrations), there was no internal equilibrium and the +/+ cells went extinct. Although we did not observe complete extinction of the −/− type at low serum concentrations (Figs. 2 and 3*C*), the +/+ type could go to fixation if the cost of the growth factor was low enough, and in this case, there would be no social dilemma (23).

Adding exogenous IGF-II changed the dynamics in a similar way to increasing the amount of serum. An alternative interpretation could be that adding IGF-II reduced the amount of growth factor that must be produced by the cells to generate a certain benefit. In other words, adding exogenous growth factors reduced the value (h) of the inflection point of the benefit function (Fig. S2), thus reducing the equilibrium fraction of +/+ cells. Theory also predicted that the maximum growth rate should be observed at intermediate frequencies of producers (23), a prediction that was confirmed in our observations of the growth rates of mixed populations in vitro (Fig. 4).

Dynamics in Planar Heterogeneous Networks. Although we have assumed a well-mixed population of cells in the above arguments about conflict and cooperation, many solid tumors, including the insulinomas produced by our cells, are populations with a defined spatial structure, a detail that is known to be important in studies on the evolution of cooperation (25, 26). To verify the

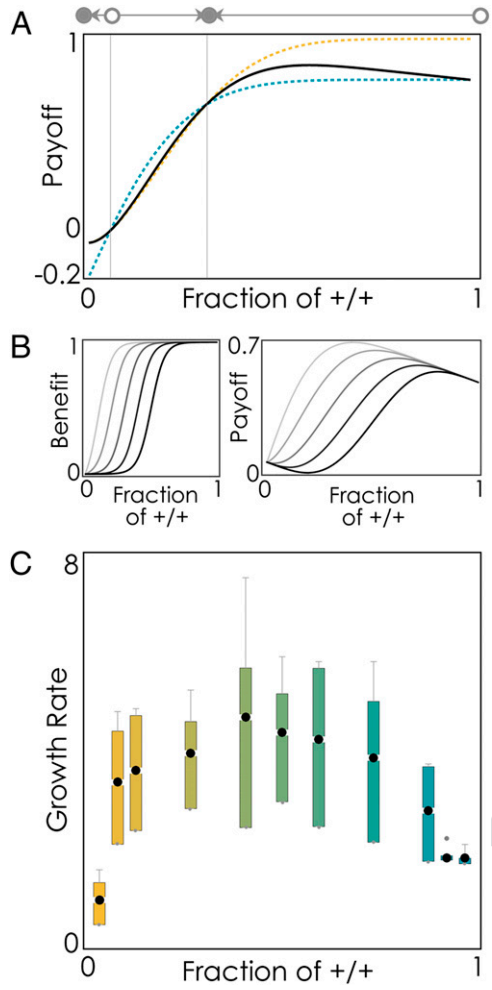

Fig. 4. Growth rates peak at intermediate frequencies of producers. (*A*) Predicted fitness of the two cell types (dotted blue curve, +/+; dotted yellow curve, −/−) and the average fitness of the population (solid black line) as a function of the frequency of +/+ cells. Circles show the equilibria (●, stable; ○, unstable), and arrows show the direction of the dynamics ($h = 0.2$, $s = 30$, $c = 0.2$, $n = 10$). (*B*) Benefit of growth factors and the corresponding predicted average tumor fitness (payoff) as a function of the frequency of +/+ cells in the population for given values of h (the position of the threshold: light to dark curves; $h = 0.1$ to 0.5, $s = 20$, $c = 0.4$, $n = 10$). (*C*) Observed growth rates of mixed cultures in vitro as a function of the fraction of +/+ cells. Boxes show the mean and the 25% and 75% quartiles (upper and lower fences, respectively).

importance of spatial structure on the dynamics, we ran simulations of evolution in spatial planar networks, in line with previous models of cooperation in spatially structured populations, with the difference that monolayers of cells, being heterogeneous planar networks, were modeled as Voronoi graphs (Fig. 5*A* and *SI Materials and Methods*). Voronoi graphs resemble the distributions of polygons in cell tissues (27) rather than regular lattices (in which all nodes have the same number of neighbors) or a scale-free network (which is not planar), which are the two topologies generally used in the study of cooperation in social networks (25, 26).

Simulations of the evolution of growth factor production on Voronoi networks showed that the +/+ and −/− cells can coexist in stable equilibrium (Fig. 5 *B*–*D*). Similar to what happens in well-mixed populations, increasing the diffusion range of the growth factors reduces the amount of producer cells and the overall fitness of the populations. Cooperation is more efficient when the cost/benefit ratio of producing the growth factor is lower and when intermediate levels of producers are required (i.e., when the benefit function has an inflection at intermediate frequencies of producers) (Fig. 5*D*). Increasing the diffusion range d of the growth factor reduces the fraction of +/+ cells, and therefore the growth rate of the tumor (Fig. 5*D*), because it increases the size of the group that benefits from a cell's production. Whereas the overall fraction of +/+ and −/− cells remains relatively stable after a period of adjustment (Fig. 5*C*), the position of the +/+ and −/− clusters continues to change over time (Fig. 5*B* and Fig. S3). Our simulations show that the dynamics are not significantly affected by cell density (Fig. S4) or by the frequency of cell passaging during culture (Fig. S5).

Discussion

Overall, our experimental results are in line with the predictions of models of nonlinear public goods in the framework of evolutionary

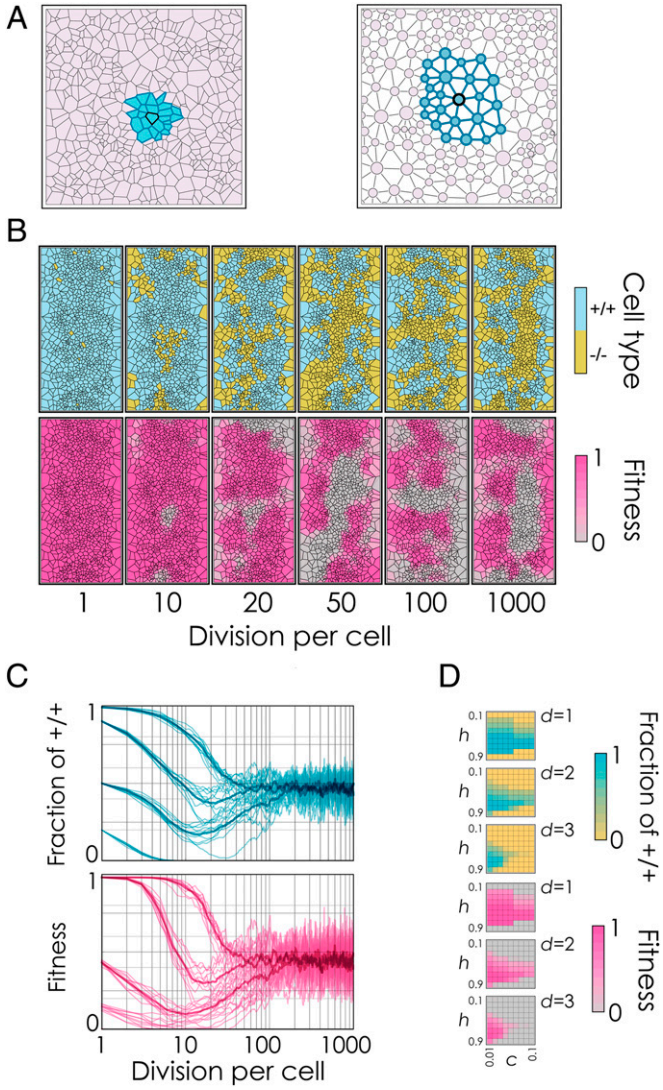

Fig. 5. Growth factor production as a public goods game on a network. (*A*) Cells in a monolayer occupy the nodes of a planar heterogeneous graph. The number of edges within the diffusion range d of the growth factor defines the interaction group [here, $d = 2$ (blue cells)]. (*B*) Snapshots of simulations in which −/− cells invade a population of +/+ cells ($d = 3$, $c = 0.02$, $h = 0.5$, $s = 20$). (*C*) Changes in the fraction of producer cells (+/+) and in tumor fitness over time in simulations (thick lines are the average of 10 simulations). (*D*) Fraction of +/+ cells and fitness at equilibrium as a function of the inflection point h and of the cost of production c for different values of the diffusion range d.

game theory (23, 25, 26): Stable coexistence of producers and nonproducers (and therefore stable heterogeneity) can be maintained if the effect of the growth factor is a nonlinear function of the frequency of producers. Although cooperation requires positive assortment in linear public goods games, and the dynamics in spatially structured populations are therefore significantly different from the dynamics in well-mixed populations (25, 26), in the case of nonlinear benefits, cooperation is maintained in both well-mixed and spatial populations by frequency-dependent selection. Strict interdependence between subclones (10, 11) is not the case in our system, and, as we have shown, it is not necessary to maintain heterogeneity if the effect of the growth factor is nonlinear.

Because the fraction of producers is directly proportional to the concentration of the growth factor, our results imply that stable heterogeneity can be maintained if the benefit of a growth factor is a nonlinear function of its concentrations. Although we have focused on IGF-II, cooperation for the production of diffusible factors is probably common in cancer cell proliferation and in other processes that require diffusible molecules, such as sustained angiogenesis, immune system evasion, and metastasis (10). Collective effects have been previously reported for acidic FGF-1 in bladder carcinoma cells (28). Nonlinear effects are likely to be the rule for most biological molecules; the nonlinearity is generally a sigmoid shape described by the Hill equation (29), and examples of sigmoid effects have been reported for IGF-II and other growth factors (24, 30, 31).

Our results are related to studies of cooperation and competition in microbial cell populations where examples of cooperation and defection for the production of public goods have been observed (13–15). Similar to what happens for antibiotic resistance in microbial populations (32), which is promoted by the production of diffusible public goods (14), cooperation between tumor cells is an obstacle for therapies that target growth factors. Reducing the amount of circulating growth factors may lead to a reduction in tumor growth in the short term, but it will also increase the inflection point of the benefit function (the number of producer cells necessary to achieve a benefit), thus simply shifting the equilibrium to a higher fraction of +/+ cells, which may potentially explain the relapse observed in patients treated with therapies targeting growth factors (33). On the other hand, modifying the dynamics of the production of growth factors, by increasing their diffusion range for instance, might lead to a stable reduction of tumor proliferation.

Although the view that cancer is an evolutionary process (1, 2) is now widely accepted (3, 4), and the importance of understanding its dynamics has been recognized (32, 34), evolutionary methods are still largely neglected in the study of resistance to anticancer therapies (35). Our results suggest that further work on the dynamics of "social" interactions among cancer cells may reveal further insight into the dynamics of cancer, and hopefully guide research toward evolutionarily stable therapies (36).

Materials and Methods

Cell Lines. β-tumor cell lines were derived from WT Rip1Tag2 mice (+/+) (16) and from the same transgenic mice carrying a homozygous deletion of the IGF-II gene (–/–) (17). The cell lines were maintained in culture in DMEM supplied with 10% FBS, 1% glutamine, and 1% antibiotics. Conditioned medium was obtained from subconfluent cultures kept for 48 h in DMEM supplied with 5% FBS, 1% glutamine, and 1% antibiotics.

Proliferation Assay. A total of 30,000 cells were plated per well in 24-multiwell plates. After treatment, cells were fixed with 2.5% glutaraldehyde dissolved in PBS for 30 min at room temperature (RT). After washing twice with deionized water, crystal violet 0.1% solution in 20% methanol was added in each well for 15 min. Afterward, the solution was removed and each well was washed with water and allowed to dry at RT. The color was dissolved in 50 μL of 10% acetic acid solution and transferred in 96-multiwell plates, and intensity was measured by a plate reader at 595 nm. The growth rate is defined as the relative change in density during the log phase (after 10 d).

Measuring Frequencies by Flow Cytometry. The producer (+/+) type was stably transduced by lentiviral infection with a pLenti-EGFP plasmid and selected for EGFP expression by puromycin (2 ng/mL) treatment for 48 h. The producer (+/+) type therefore expresses EGFP constitutively. We measured the fraction of the two types in mixed populations using an Accuri C6 flow cytometer (Becton Dickinson) (488-nm excitation, 533-nm emission, 300-nm BP, FL1 emission filter) after gating out cellular debris and selecting only single cells for analysis. We typically counted 50,000 cells.

Public Goods Game. A cell can be a producer (+/+) or a nonproducer (–/–) of IGF-II. Producers pay a fixed cost c that nonproducers do not pay. A cell benefits from the IGF-II produced by all of the cells in its group of size n. We assume that the benefit function has a sigmoid shape. The benefits for +/+ and –/– cells are therefore, respectively, the normalized versions of $V(j+1)$ and $V(j)$, where $V(j) = \beta/[1+e^{-s(j/n-h)}]$. We assume that $\beta = 1$ and $0 < c < 1$, j is the number of +/+ cells among the other $n-1$ cells, h defines the position of the inflection point ($h \to 1$ gives strictly increasing returns, and $h \to 0$ gives strictly diminishing returns), and s defines the steepness of the function at the inflection point ($s \to \infty$ models a threshold public goods game, $s \to 0$ models an N-person prisoner's dilemma) (23).

Evolutionary Dynamics on Networks. In well-mixed populations, groups of size n are updated at every generation. In spatially structured populations, group size n is a function of the diffusion range d of the growth factor, measured as the lowest number of edges (the shortest distance) between nodes (Fig. 5*A*). The spatial network is a Voronoi graph obtained by a Delaunay triangulation of random points on a sphere (to avoid distortions due to edge effects) (*SI Materials and Methods*). The average connectivity is six, with a unimodal distribution; nodes with fewer than four or more than eight connections are rare (27). Individual cells occupy the nodes of a network of size 1,000. The process starts with a number of nonproducers placed at random on the graph. At each game round, strategies are updated according to a standard death–birth process in which the probability that a node reproduces is proportional to its fitness (25, 26) (*SI Materials and Methods*). Results are obtained by averaging the final 20% of 1 million generations, averaged over 10 runs.

ACKNOWLEDGMENTS. We thank Dieter Ebert, István Scheuring, Dylan Edwards, and Stephen Robinson for comments on the manuscript; Stephen Robinson, Aleksander Gontarczyk, and Linh Le for help with cell culture; and Ernesta Fagiani and Darren Sexton for help with flow cytometry. This work was supported by Natural Environment Research Council Grant NE/H015701/1, the Swiss National Science Foundation, EU-FP7 TuMIC HEALTH-F2-2008-201662, and the SystemsX.ch RTD project Cellplasticity.

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Supporting Information

Archetti et al. 10.1073/pnas.1414653112

SI Materials and Methods

Public Goods Game. A cell can be a producer (+/+) or a nonproducer (−/−) of a growth factor. Producers pay a fixed cost c that nonproducers do not pay. All cells (+/+ and −/−) benefit from the public good produced by all of the cells in their group of size n (in spatially structured populations, n depends on the diffusion range of the factor, as discussed below). Fitness is therefore a frequency-dependent function of the number of producer cells j. We model this function using the logistic function

$$V(j) = \frac{\beta}{1 + e^{-s(j/n-h)}},$$

where β is the maximum benefit produced by the growth factor; we can assume, without loss of generality, that $\beta = 1$ and $0 < c < 1$. The parameter h controls the position of the inflection point ($h \to 1$ yields strictly increasing returns, and $h \to 0$ yields strictly diminishing returns), and the parameter s controls the steepness of the function at the inflection point ($s \to \infty$ yields a Heaviside step function, and $s \to 0$ yields a linear function). We define the benefit function $b(j)$ of IGF-II as the normalized version of $V(j)$:

$$b(j) = \left[V(j)\text{-}V(0)\right] / \left[V(n)\text{-}V(0)\right].$$

The only notable effect of the normalization is that, for $s \to 0$, it makes the benefit function an increasing rather than a constant linear function, enabling us to model, in addition to the sigmoid function commonly observed in biological molecules (1), the threshold public goods game ($s \to \infty$) and the N-person prisoner's dilemma ($s \to 0$) commonly used in multiplayer public goods games (2).

Fitness in Well-Mixed Populations. The initial cultures are well-mixed populations on the day the cells are plated. In an infinitely large population, the average payoffs of +/+ and −/− cells can be written as, respectively,

$$W_{+/+} = \sum_{j=0}^{n-1} \binom{n-1}{j} x^j (1-x)^{n-1-j} \cdot b(j+1) - c$$

$$W_{-/-} = \sum_{j=0}^{n-1} \binom{n-1}{j} x^j (1-x)^{n-1-j} \cdot b(j),$$

where j is the number of +/+ cells among the other $n-1$ cells in the group. In a finite population, sampling of individuals follows a hypergeometric distribution and the average payoffs of +/+ and −/− can be written as, respectively, (3)

$$W_{+/+} = \binom{Z-1}{n-1}^{-1} \sum_{j=0}^{n-1} \binom{i-1}{j} \binom{Z-i}{n-j-1} \cdot b(j+1) - c$$

$$W_{-/-} = \binom{Z-1}{n-1}^{-1} \sum_{j=0}^{n-1} \binom{i}{j} \binom{Z-i-1}{n-j-1} \cdot b(j),$$

where i is the number of +/+ individuals in the population of size Z. Assuming a stochastic birth–death process combined with a pairwise comparison rule, two individuals from the population, A and B, are randomly selected for update. The strategy of A will replace the strategy of B with a probability given by the Fermi function, $p \equiv 1/[1 + e^{-\beta(W_A - W_B)}]$, and the reverse will happen with probability $1 - p$. The quantity corresponding to the "gradient of selection" in the replicator dynamics is given in finite populations by (3)

$$g(i) = \left[i(Z-i)/Z^2\right] \tanh\left[\beta\left(W_{+/+} - W_{-/-}\right)/2\right].$$

The quantity β specifies the intensity of selection (for $\beta << 1$, selection is weak, and for $Z \to \infty$, one recovers the case of infinite populations).

Network Topology. The two topologies usually considered in the study of spatial games, regular lattices (in which all individual nodes are topologically equivalent) and scale-free networks (in which different individuals have a distinct number of connections) (4, 5), are not appropriate for the study of monolayers of cells because they either neglect the importance of variation in connectivity (regular lattices) or are not planar (scale-free networks). We therefore use Voronoi networks.

A Voronoi diagram (tessellation) of a set of nodes is a collection of convex polygons, each corresponding to one of the nodes, with all of the points in one polygon being closer to the corresponding node than to any other node. The boundary between two adjacent polygons is a line segment, and the line that contains it is the perpendicular bisector of the segment joining the two nodes. A Voronoi network is defined as such node-joining segments. The average connectivity of Voronoi networks is six, with a unimodal distribution; nodes with fewer than four or more than eight connections are rare. The average group size for $d = 1$ (average neighborhood size) is therefore seven. Such a structure resembles the distribution of polygons in cell tissues (6, 7).

More specifically, the Voronoi diagram of V is a subdivision of space into Voronoi cells; for any vertex i belonging to V, the Voronoi cell of i is the set of points with a distance to i not greater than to any other vertex of V. The dual of the Voronoi diagram is the Delaunay triangulation defined on the same vertex set (Voronoi polygons correspond to Delaunay vertices). Two-dimensional Voronoi graphs are obtained by a Delaunay triangulation of random points, using the DelaunayTriangulation implementation in *Mathematica* 8 (Wolfram Research, Inc.). If the points are drawn on a circle or on a square, the implementation is straightforward. Points can also be drawn on a sphere to avoid edge effects (this ■■■ is equivalent to the common procedure of connecting the edges of a regular lattice to form a toroidal network). Points on a sphere are defined by colatitude (φ) and longitude (θ), both of which are drawn from a uniform distribution with support [0,2π]. These points can be mapped into the Cartesian space using the standard transformation $x = r * \sin\varphi\cos\theta$, $y = r * \sin\varphi\sin\theta$, $z = r * \cos\varphi$, with $r = 1$. To reduce the density of points around the poles, making them evenly distributed over the spherical area, we define φ as 2ArcSin[Sqrt[Random[0,1]]] using the "Inverse CDF Method" in *Mathematica* rather than simply drawing it from [0,2π]. In the Cartesian space (the figures used here), the polygons appear distorted at the edges, and they are actually connected with the polygons on the opposite edge to form a sphere (as in a geographic projection of the Earth).

Diffusion and Group Size in Spatially Structured Populations. In well-mixed populations, groups of size n are updated at every generation. In spatially structured populations, group size n is a function

of the diffusion range d of the growth factor, where d is the lowest number of edges (the shortest distance) between nodes ($d = 1$ defines the one-step neighbors of a node). A standard assumption in the study of public goods games on networks is that an individual's action affects only the fitness of individuals one node away: Each individual belongs to n different groups, with each group centered on one of that individual's one-step neighbors, and an individual's fitness is the sum of all of the payoffs accumulated in all of the groups that individual belongs to (5). Although these assumptions are reasonable for interactions in human social networks, this ■■■ is not the case for cellular networks, because growth factors produced by cells in a tissue typically diffuse beyond a cell's neighbors; the benefit a cell gets as a result of available growth factors is a function of the number of producer cells within the diffusion range of the factor, not just of the number of producers in the upgrade neighborhood (one-step neighbors) or of all of the individuals belonging to the neighbors' groups. In other words, to study diffusible public goods, we must decouple the interaction neighborhood (the group playing the public goods game, defined by the diffusion range d) and the update neighborhood (the one-step neighbors) (8–13).

Evolution in Spatially Structured Populations. In spatially structured populations, the process starts with a number of nonproducers (−/−) placed at random on the graph in which all other nodes are occupied by producers (+/+). At each round, strategies are updated according to the following rule (5): A node x with a payoff P_x is selected (at random) for update (death), and a node y (with a payoff P_y) is then chosen among x's neighbors. If $P_x < P_y$, x will adopt y's strategy (unconditional imitation); in the stochastic case, replacement occurs with a probability given by $(P_y - P_x)/M$, where M ensures the proper normalization, and is given by the maximum possible difference between the payoffs of x and y. Results are the average of the final 200,000 of 1 million generations, averaged over 10 different runs. In monolayers of cells in vitro, cells are initially not confluent, and may therefore lack one-step neighbors; we model nonconfluent cultures as networks in which nodes corresponding to missing cells have a fitness equal to zero. Transferring (passaging) cells in vitro is equivalent to updating periodically with a new random network the spatial structure of the populations every g divisions per cell; $g = 1$ corresponds to a well-mixed population, and $g = 1$ million corresponds to a population with a fixed spatial structure.

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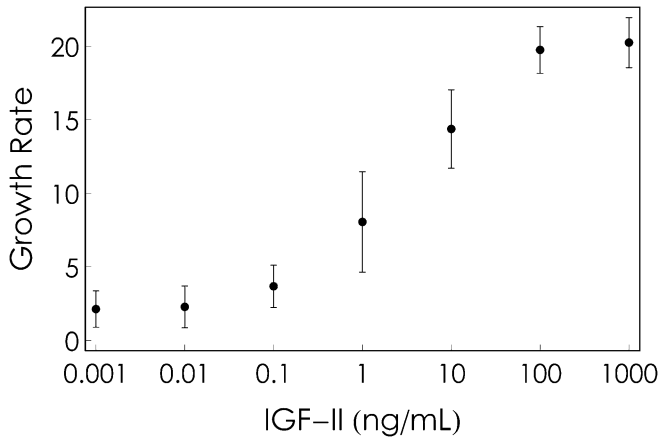

Fig. S1. Cell proliferation is a sigmoid function of the concentration of IGF-II. The growth rates of –/– cells cultured in medium supplemented with different amounts of IGF-II are shown. Error bars represent SD.

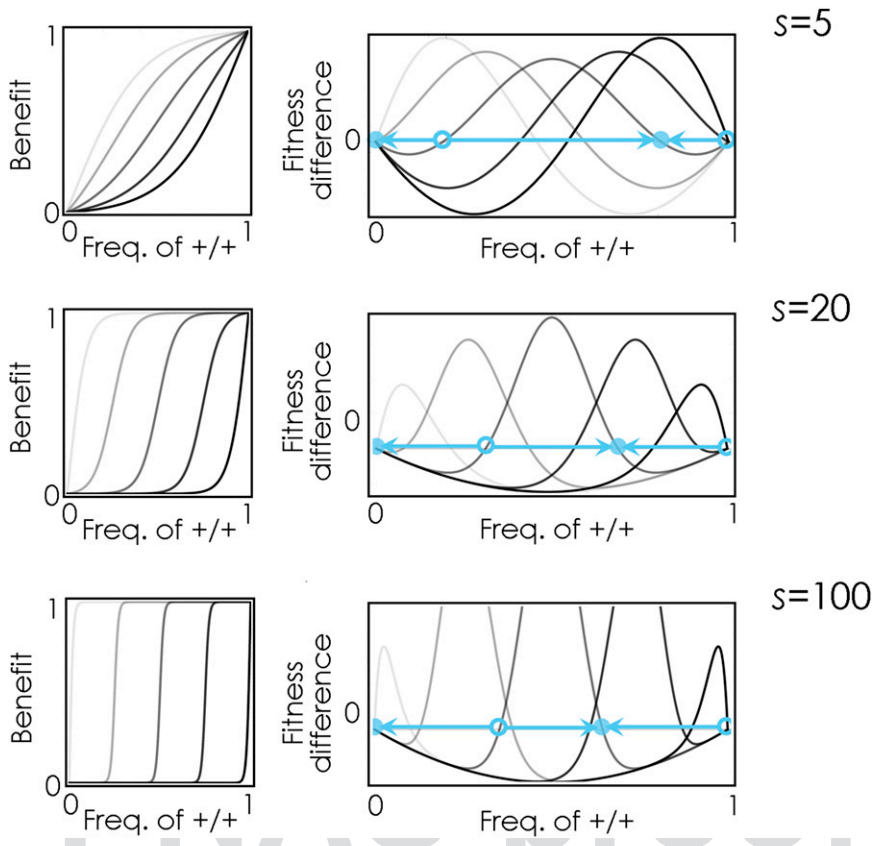

Fig. S2. Evolutionary dynamics and equilibria of nonlinear public goods production. The benefit due to growth factors and the predicted fitness difference between +/+ and −/− cells as a function of the frequency (Freq.) of +/+ cells in the population for given values of s (the steepness of the benefit function) and different values of h (the position of the inflection point: 0, 0.25, 0.5, 0.75, 1; curves from light to dark gray) are shown. The difference in fitness between +/+ and −/− cells determines the dynamics: Where it is positive, +/+ cells increase in frequency, and where it is negative, they decrease in frequency; equilibria occur where it is zero (shown here only for the intermediate value $h = 0.5$: ●, stable; ○, unstable; arrows show the direction of change). $\beta = 1$, $Z = 1{,}000$, $d = 3$, $c = 0.02$.

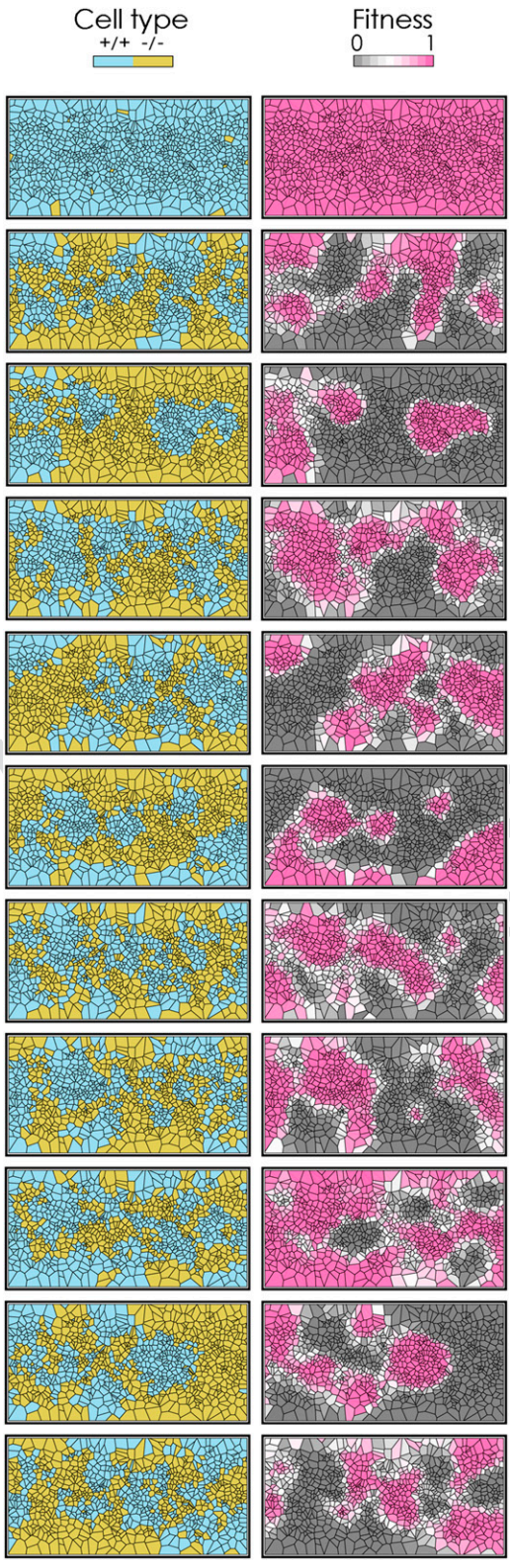

Fig. S3. Coexistence of producers and nonproducers. Snapshots of simulations of the spatial structure of a monolayer of cells at every 100 cell divisions. c = 0.02, h = 0.5, s = 20, d = 3.

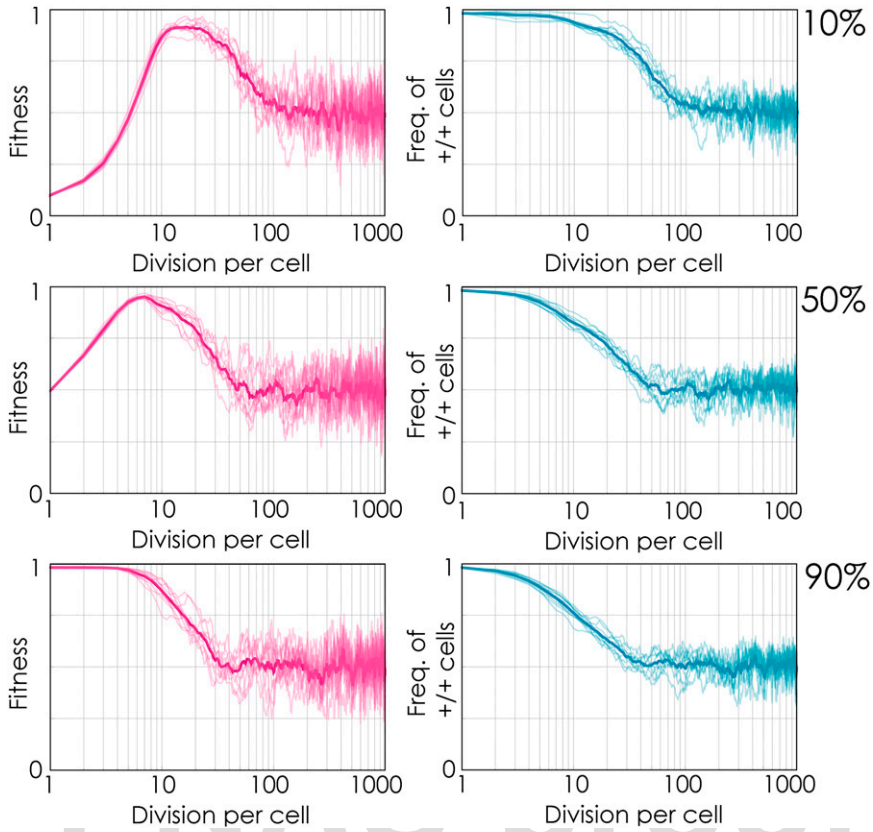

Fig. S4. Effect of cell density. Changes in the fraction of +/+ and tumor fitness over time in simulations of sparse to dense cultures (starting from 10%, 50%, or 90% confluent populations). $d = 3$, $c = 0.02$, $h = 0.5$, $s = 20$.

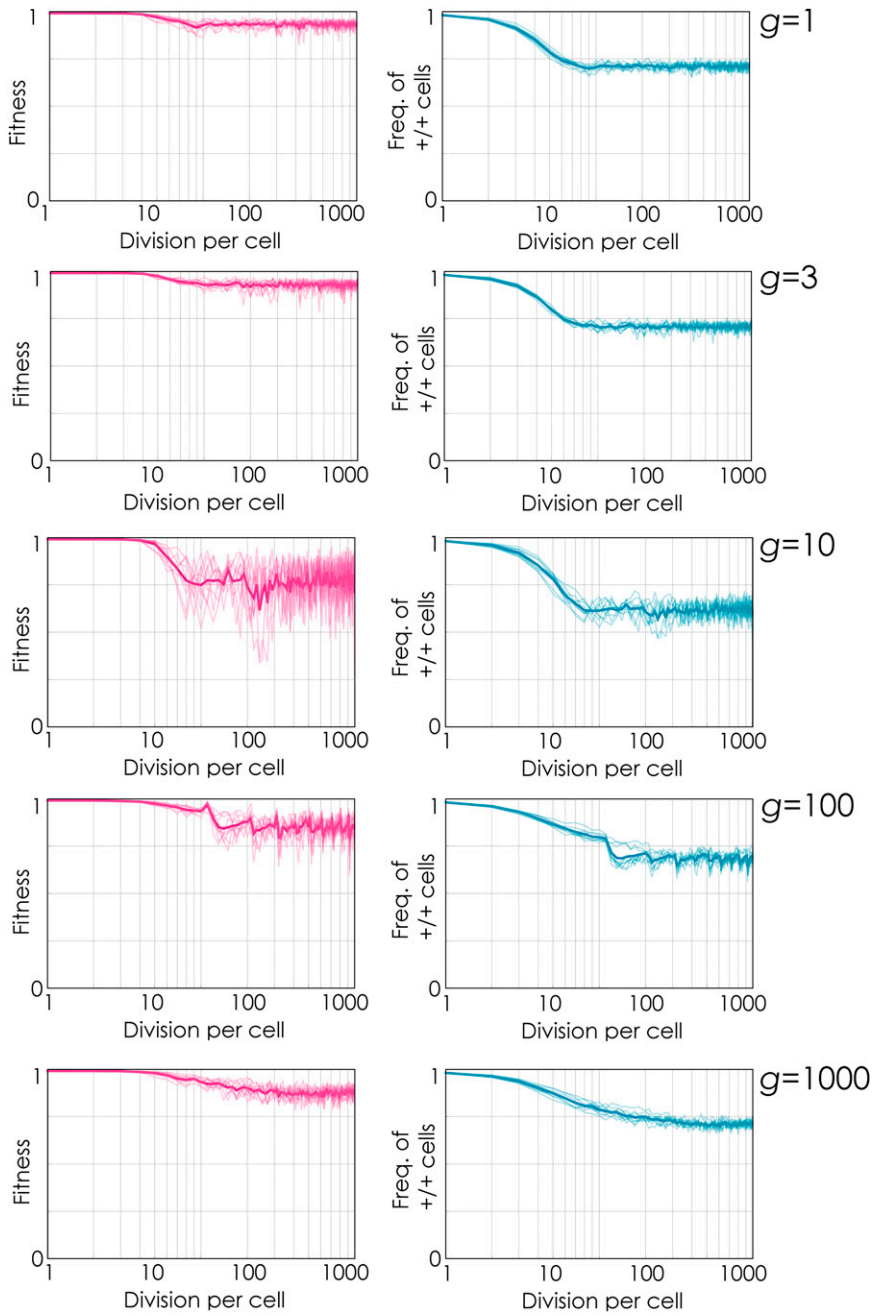

Fig. S5. Effect of passaging cells. Changes in the fraction of +/+ and tumor fitness over time in simulations of populations whose spatial structure is updated every g divisions per cell ($g = 1$ corresponds to a well-mixed population, $g = 1{,}000$ corresponds to a population with a constant spatial structure). $d = 1$, $c = 0.01$, $h = 0.5$, $s = 20$.