

Research Paper

The Emergence of Tumor Metastases

David Dingli^{1,2,*}

Franziska Michor^{3,4}

Tibor Antal¹

Jorge M. Pacheco^{1,5}

¹Program for Evolutionary Dynamics; Harvard University; Cambridge, Massachusetts USA

²Division of Hematology; Mayo Clinic College of Medicine; Rochester, Minnesota USA

³Harvard Society of Fellows; Cambridge, Massachusetts USA

⁴Dana Farber Cancer Institute, Boston, Massachusetts USA

⁵CFTC and Departamento de Física da Universidade de Lisboa; Complexo Interdisciplinar; Lisboa, Portugal

*Correspondence to: David Dingli; Program for Evolutionary Dynamics, Harvard University; One Brattle Square; Cambridge, Massachusetts 02138 USA; Tel.: 617.496.5543; Fax: 617.496.4629; Email: dingli@fas.harvard.edu

Original manuscript submitted: 10/23/06
Manuscript accepted: 12/16/06

Previously published online as a *Cancer Biology & Therapy* E-publication:
<http://www.landesbioscience.com/journals/cb/abstract.php?id=3720>

KEY WORDS

Tumor, metastases, mathematical modeling, dynamics, evolution

ACKNOWLEDGEMENTS

We would like to thank Professor Martin A. Nowak for many helpful suggestions and encouragement as well as critical reading of the manuscript. Financial support from Mayo Foundation (D.D.), the Harvard Society of Fellows (F.M.) and FCT-Portugal (J.M.P.) is gratefully acknowledged. The Program for Evolutionary Dynamics is supported by Jeffrey Epstein.

ABSTRACT

The appearance of metastases is an ominous sign in the natural history of any malignant tumor. Their presence implies a high tumor burden and greatly decreases the probability of a cure. Metastasis development requires the evolution of tumor cells that can survive in an environment that is normally not supportive to their growth and such cells must leave the tumor to establish tumor niches elsewhere. The interactions between the appearance of cells with metastatic ability in the primary tumor and their exit from the tumor lead to complex dynamics that can be either beneficial or detrimental to the tumor. We develop a simple mathematical model to illustrate how the interplay between mutation rate and export probability affects the intratumoral dynamics of metastasis-enabled cells and the rate of metastases formation.

INTRODUCTION

Cancer is a leading cause of morbidity and mortality in many countries. With the exception of most hematopoietic neoplasms that are by definition diffuse diseases from the time of diagnosis, solid tumors in general start at one particular site (primary tumor). The tumor can cause symptoms due to localized growth but often it is clinically silent and discovered due to metastatic spread. With the development of metastases, the potential for cure, with rare exceptions (e.g., germ cell tumors, ref. 1), is greatly diminished and most patients die relatively rapidly from progressive disease.² Even in the absence of clinically or pathologically overt metastases at diagnosis, patients often develop metastatic disease, leading to the concept of micro-metastases that are too small to be detected by current diagnostic technologies.^{3,4} Therefore, patients are often given adjuvant therapy after curative surgery to minimize the risk of loco-regional recurrence as well as metastatic disease. The efficacy of this approach has been well documented with breast,⁵ colon⁶ and lung carcinoma.⁷

Although there is a trend for a higher risk of metastases as the size of the primary tumor increases, this is not always the case. Indeed, the concept of unknown primary in the presence of diffuse metastases is well known.^{8,9} Moreover, the kinetics of the development of metastatic disease is highly variable.^{10,11} Various theories have been proposed to explain this behavior including tumor cell dormancy,¹² inhibition of angiogenesis¹³ and interactions with the immune system.¹⁴

Mathematical models have enhanced our understanding of tumor dynamics and metastases formation and suggest ways to optimize therapy. The fractal heterogeneity of blood flow has been implicated as a determining factor in the distribution of metastases.¹⁵ In breast cancer, modeling of the number of involved axillary lymph nodes can estimate the risk of metastases and prognosis.^{16,17} The interaction between tumor cells and their surrounding stroma describes four broad behaviors observed in tumors, namely latency, complete regression, indefinite growth and metastases formation.¹⁸ A model of prostate cancer suggests that the presence of diffuse metastases is related to an aggressive primary tumor more than to the infiltration of loco-regional lymph node at the time of diagnosis, implying that aggressive local control could decrease the incidence of metastases.¹⁹ These reports are part of an increasing body of literature that addresses the quantitative and kinetic aspects of carcinogenesis.²⁰⁻³²

Cells that undergo mitosis acquire novel mutations, some of which may lead to the acquisition of the metastatic phenotype.³³ Such a phenotype is a property of a given tumor cell, but the tumor is composed of a heterogeneous population of cells,³³ and the evolution of the whole population has to be studied. Indeed, the tumor need not be

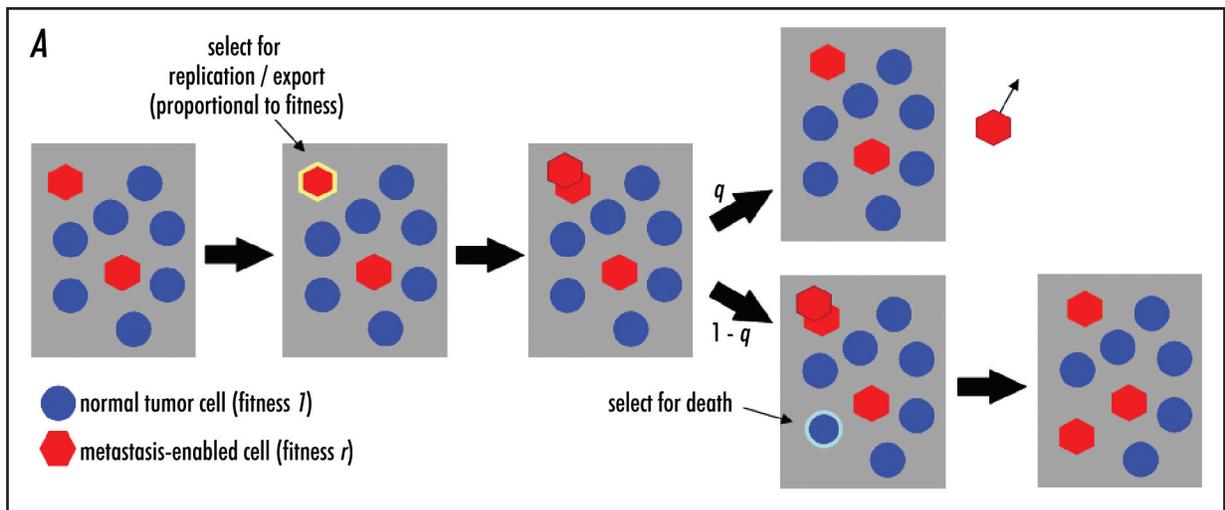


Figure 1. Model A: Stochastic model of tumor cell dynamics. At any given time, we consider two types of cells in the tumor: normal tumor cells (circles) and tumor cells which acquired metastatic ability via mutation (hexagons). The mutation probability is μ per replication event. Normal tumor cells have relative fitness 1, while metastases enabled cells have fitness r . At each step, a cell is chosen for replication proportional to fitness. If the chosen cell is of normal type, it will produce offspring which will replace a randomly chosen cell that dies. If the chosen cell is of mutated type, then with probability q one cell will leave the tumor to form metastases elsewhere whereas with probability $(1 - q)$ it will remain in the tumor. In the latter case, it replaces a randomly chosen cell that dies. The total number of cells in the tumor remains strictly constant at all times.

homogenous for metastatic cells to leave the primary site and seed additional niches.³³ In this report, we explore mathematical models of metastasis development that consider coupling between acquisition of the metastatic phenotype with a finite probability that the daughter cell will exit the primary tumor. Tumor cells with metastatic potential leave the primary tumor at a rapid rate but form viable metastases inefficiently.³⁴ Our models include a mechanism for cells to leave the primary tumor site and attempt to form metastasis elsewhere. For the sake of simplicity, we initially assume that the tumor cell population tends to remain constant. This scenario describes situations where tumor cells have reached a constant abundance and need to accumulate further mutations to initiate the next wave of clonal expansion. Subsequently, we explore models where the tumor cell population grows exponentially. We obtain rich dynamics resulting from the interplay between the rate of cell replication and the probability of tumor cells to leave the tumor after acquiring the metastatic phenotype. This paper represents an extension of earlier work on the dynamics of metastasis-enabled cells in a population of constant size.^{35,36}

MATERIALS AND METHODS

Models. Consider a population of N tumor cells proliferating according to a stochastic process. Tumor cells have a given fitness r_T (without loss of generality, we set $r_T = 1$). A cell mutated with respect to the metastasis-enabling gene has fitness r and we assume that initially all tumor cells are wild type - they have not yet evolved metastatic ability. At each elementary time step, one tumor cell is randomly chosen for replication proportional to its fitness, producing a daughter cell (birth event). At every replication, a cell undergoes a mutation with probability μ ;^{33,37} this mutation enables it to metastasize and confers to it a relative fitness r . If $r = 1$, the mutation is neutral and the mutant cell has the same net reproductive rate as nonmutated cells. When $r > 1$, the mutant has a fitness advantage and if $r < 1$, the mutant is at a disadvantage compared to nonmutated cells. Simultaneously, a cell may be chosen for death with uniform

probability (death event). When this happens, the population remains constant. In these models we neglect back-mutation.

After N replication/death events, the population has undergone a typical generation, which is (inversely) proportional to the rate of cell replication. This naturally sets the time scale of our evolutionary dynamics: we measure the time in generations. Furthermore, tumor cells which have acquired the metastatic phenotype via mutation may leave the tumor to form metastases elsewhere. We model this as a stochastic process, attributing to this cell type a probability q to leave the tumor (export event), once selected for replication. We discuss two different models, which describe tumor dynamics at different levels of complexity, via the interplay between birth, death and export events.

Model A—Constant tumor size. This model combines birth, death and export events while maintaining the total tumor population size at $N = \text{constant}$. At a given time t , there are $M < N$ mutated cells. Then, the probability of choosing a mutated cell for reproduction is given by

$$p_M = \frac{rM}{rM + N - M},$$

whereas the probability to select a normal cell for replication is $p_N = 1 - p_M$. The stochastic dynamics always starts with a birth event, leading to the replication of the chosen cell. If the chosen cell is a normal tumor cell, with probability μ the daughter cell acquires a metastasis enabling mutation. If the chosen cell is a metastasis enabled cell, a new daughter cell of the same type is produced, and two scenarios are possible (Fig. 1): with probability q the daughter cell is exported such that it attempts to establish a metastasis elsewhere and, with probability $(1 - q)$, the daughter cell stays within the

¹ Since the mutation probability μ is much smaller than one, this model leads to the same results as a model in which the export event is not linked to the birth event, but instead, is integrated in the death event. In such a case, cells should not be selected for death with uniform probability; instead, a metastasis enabled cell should be chosen for death with probability $p_d = (1 + q)M / [(1 - q)M + 1(N - M)]$, being subsequently exported with probability q .

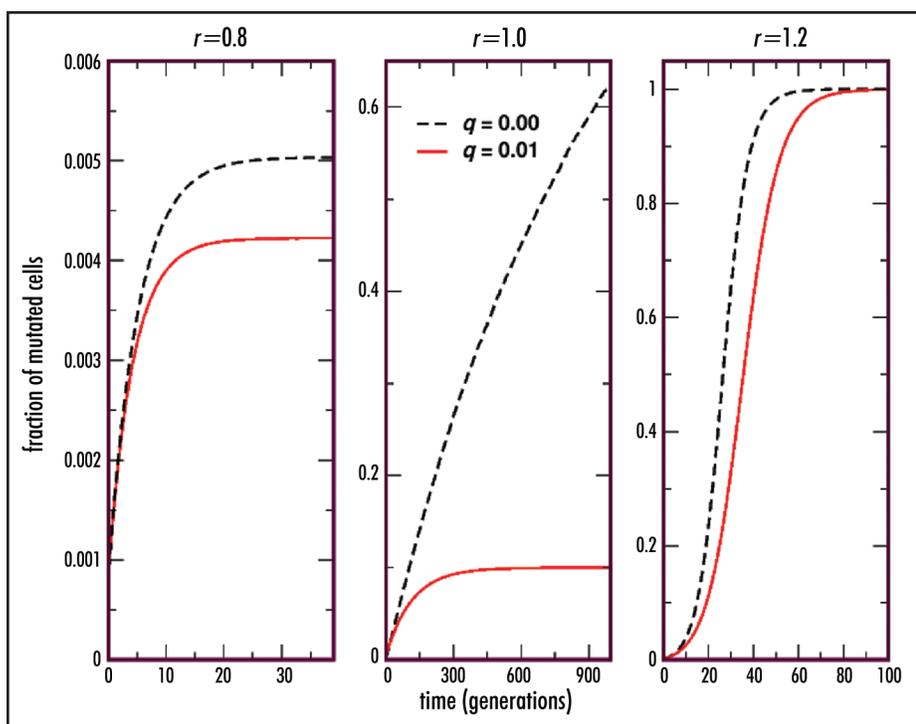


Figure 2. Model A: The fraction of metastasis enabled cells in the primary tumor is plotted as a function of evolutionary time in generations (one generation is the time required for N cells to replicate - in other words, 1 generation is the inverse of cell replication rate). In each panel two lines are drawn: the dashed line corresponds to the limit $q = 0$ whereas the solid line corresponds to the case when $q = 0.01$. The left panel illustrates the evolutionary regime whenever metastasis enabled cells are disadvantageous mutants with relative fitness $r = 0.8$. The central panel displays the dynamics for neutral mutants ($r = 1.0$) whereas the right panel shows the dynamics for advantageous mutants ($r = 1.2$). Population size is $N = 10^4$ and mutation probability is $\mu = 10^{-3}$ per replication. The effect of a finite export probability is most pronounced for neutral mutations, where the fraction of mutated cells is clearly hindered reaching a quasi-stationary regime at approximately 10%, as opposed to the $q = 0$ limit where fixation of mutated cells occurs. For mutations conferring a fitness advantage to the metastatic cells, the results indicate that the dynamics are not significantly altered (cf. main text for details).

tumor. If no export takes place, then a death event follows, such that the overall population size remains strictly constant.¹

Model B—A growing tumor. Tumor development is associated with an increase in the number of tumor cells. We model this process by introducing exponential expansion of the primary tumor in the previous model, calling this model *B*. At every step, a birth event takes place with probability b and a cell death event with probability d . The same birth and death events as in model *A* are used but since their occurrence is stochastic with $b > d$, the number of cells is not conserved and the tumor grows. Since the dynamics are intrinsically stochastic, extinction of the tumor is possible during the initial stages of tumor growth. (For the purposes of the model, we discard the results associated with stochastic extinction, and carry out 1000 simulations for each set of parameters. We maintain the mutation rate at $\mu = 10^{-3}$, and study the evolution of the tumor population up to a threshold value of $N_{\text{threshold}} = 10^7$.)

RESULTS

Model A—Stationary tumor. Whenever $q = 0$, model A maps into the branching process studied by Michor et al.³⁵ In such a case, cells that acquire metastatic capacity will always contribute to increase the fraction of mutated cells in the primary tumor population,

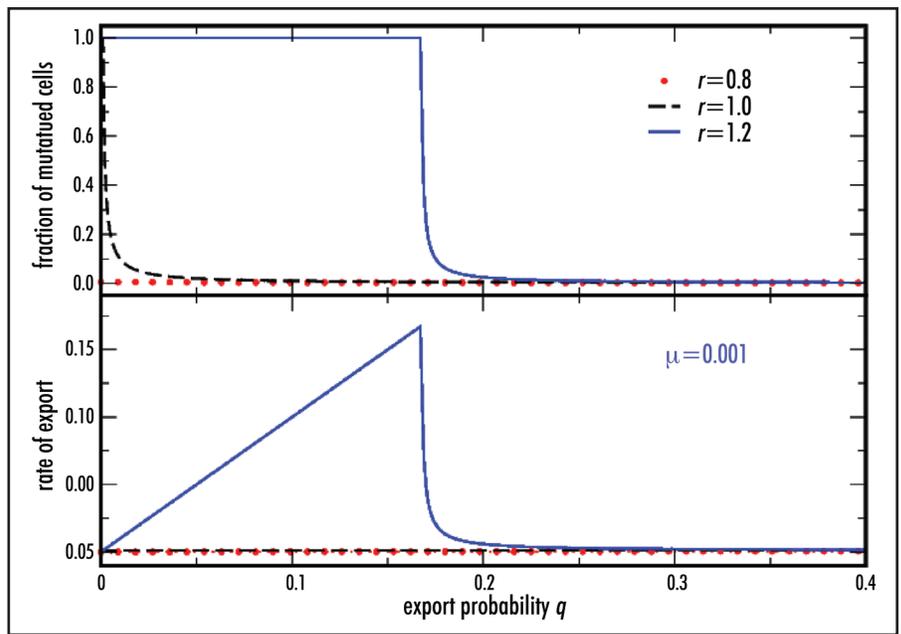
facilitating their subsequent growth.³⁵ For positive q , however, cells may leave the tumor and thus reduce the fraction of mutated cells in the tumor. As a result, there is a reduction in their growth efficiency compared to the $q = 0$ limit. We tested different tumor population sizes, and for $N \geq 10^3$ we find that the dynamical behavior of the model becomes qualitatively independent of N . In the following, we present results for a population size of $N = 10^4$. For this population size, a mutation probability of $\mu = 10^{-3}$ per replication leads to a dynamical evolution which converges to a stationary regime in a number of generations which is of the order of the population size. This mutation probability is considered in the context of genomic instability, that is normally present with advanced tumors.³⁸ A lower mutation probability slows the process, whereas a higher mutation probability will increase the rate of approach to the stationary state (see below). In Figure 2, we plot the fraction of metastasis enabled cells which remain in the tumor as a function of time. We compute this fraction for two values of the export probability q : $q = 0.0$ (dashed line) and $q = 0.01$ (solid line). With this set of parameters, we have values similar to those estimated for the B16F1 melanoma cell line which induces metastases at a rate of 5×10^{-5} /cell replication.³⁹

The results presented provide novel insight into the role played by the relative fitness r on the overall dynamics of metastasis formation. For the selected values of N , μ and q a nonzero export probability has the highest impact for neutral mutations, where the expansion of mutated cells is clearly hindered and reaches a stationary value at approximately 10%, compared to the $q = 0$ limit where fixation of mutated cells occurs.

The results shown in Figure 2 suggest that, for a given relative fitness, there is a critical value of the export probability (q_{critical}) above which the fraction of mutated cells inside the tumor no longer increases. For export probabilities satisfying $q > q_{\text{critical}}$, the tumor population does not evolve anymore into the only *absorbing state* of this model, where metastasis enabled cells invade the entire tumor. Instead, it may become “trapped” (in the sense of spending an arbitrarily long amount of time) in a quasi-stationary regime, where the probability to increase the number of mutated cells by selection and replication is balanced by the probability of exporting that same type of cells. In the appendix we develop an analytical model to characterize the quasi-stationary regime. In particular, we obtain $q_{\text{critical}} = 1 - (1 - \mu)/r$ such that, whenever $q > q_{\text{critical}}$ the fraction m of metastasis-enabled cells is given by $m = \mu/[1 - r(1 - q)]$. As a result, metastasis enabling mutations associated with large export probabilities may be detrimental to the expansion of these cells in the tumor, and inhibit the metastatic efficiency of the tumor. Note that for $\mu \ll 1$, we have $q_{\text{critical}} \ll 1 - 1/r$, which is independent of population size and mutation probability.

The previous results demonstrate that metastatic cells with a fitness advantage do not necessarily lead to a homogeneous tumor

Figure 3. Model A: Upper panel: The fraction of metastasis enabled cells in the primary tumor is plotted as a function of the export probability q after the quasi-stationary regime defined in the main text has been reached. Lower panel: the rate of export of metastasis-enabled cells originating from the main tumor is plotted as a function of q after the quasi-stationary regime has been reached. Since $\mu \ll 1$, $q_{critical} = 0$ for $r < 1$, $q_{critical} \approx \mu$ ($r = 1$) and $q_{critical} \ll 1 - 1/r$ for $r > 1$. The three lines shown in each panel correspond to three different values of r : $r = 0.8$ (dotted red lines), $r = 1$ (dashed black lines) and $r = 1.2$ (solid blue lines). When $r = 1.2$ the behavior changes dramatically between q values below and above $q_{critical}$. Below $q_{critical}$, metastasis enabled cells invade the entire tumor and the export rate grows linearly with q . Above $q_{critical}$ a sharp decline with q is observed for both quantities. For $r = 1$ the stationary fraction of mutated cells rapidly declines with increasing μ , whereas for $r < 1$ both the stationary fraction and the export rate remain small for any nonzero value of q .



population constantly exporting metastasis-enabled cells. On the contrary, if the export probability is sufficiently high, the tumor will be able to export metastatic cells at a rate which ultimately reflects more the intrinsic mutation rate than the intrinsic fitness advantage of the mutated tumor cells. This is shown in Figure 3, where we plot both the fraction of mutated cells and the rate of cell export as a function of q (cf. appendix). Since $\mu < 1$, for $r < 1$ we have $q_{critical} = 0$, whereas for $r = 1$, $q_{critical} = \mu$, such that only for $r > 1$ does $q_{critical}$ deviate significantly from 0. Clearly, for $r > 1$ the behavior changes dramatically for values of q below and above $q_{critical}$. In particular, the export rate reaches a maximum at $q_{critical}$ followed by a sharp decline, which clearly illustrates the detrimental effect of high export probabilities on the overall efficiency of tumor metastasis formation. For $r = 1$ the stationary fraction of mutated cells rapidly declines with increasing μ , whereas for $r < 1$ both the stationary fraction and the export rate remain small for any nonzero value of q .

Model B—Growing tumor. The dynamics of a growing tumor is characterized by an exponential increase in the total number of cells. Export of cells becomes possible once the first mutation takes place. If we compute the fraction of mutated cells, $m = M/N$, as a function of time, different individual simulations may exhibit distinct patterns mainly due to the stochasticity of occurrence of mutations. Nonetheless, even when the population is growing, a dynamic quasi-stationary distribution is reached for given values of μ and q . This happens if stochastic fluctuations are small, an expected feature when the total number of cells is already large. As discussed in detail in the appendix, the fraction m of metastasis-enabled cells in this quasi-stationary regime is given by the same equation of model A: $m = \mu/[1-r(1-q)]$ whenever

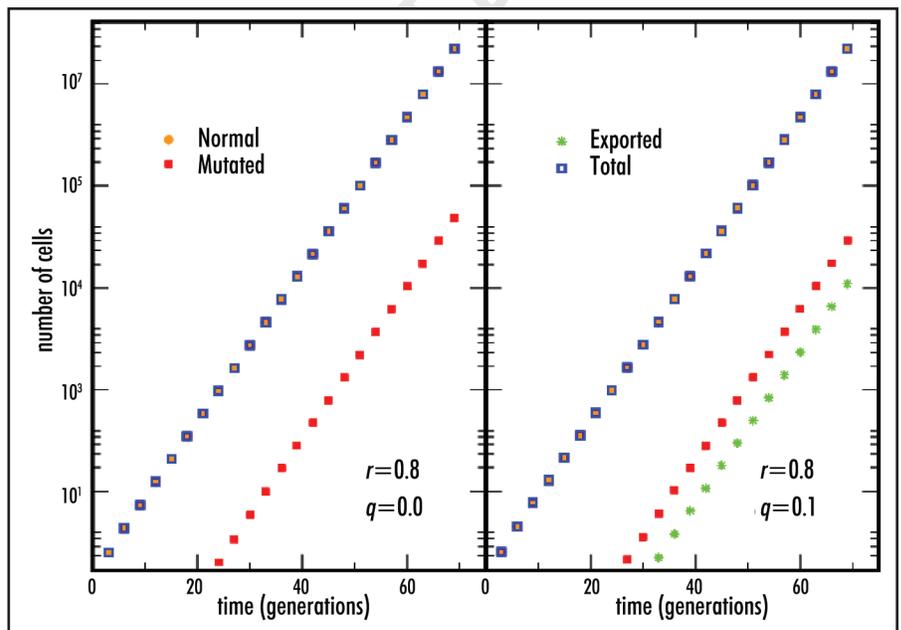


Figure 4. Model B: Dynamics of metastasis formation for a growing tumor, for $r = 0.8$, starting from a single nonmutated cell (mutation rate $\mu = 0.001$). We plot, on a logarithmic scale, the total number of nonmutated tumor cells (orange solid circles), mutated cells (red solid squares), their sum (blue open squares) and the total number of exported cells (green stars). Tumor growth is stopped when $N_{threshold} = 10^7$ is reached, and the figure shows the last 75 generations of tumor growth, where the exponential growth is associated with straight lines. Most cells in the tumor are nonmutated tumor cells, and an export probability of $q = 0.1$ mostly contributes to depleting the tumor from mutated cells. Parameter values are $b = 0.7$ and $d = 0.5$, and results correspond to an average over 1000 simulations for each set of parameters.

$q > q_{critical}$ where $q_{critical}$ is again given by $q_{critical} \ll 1 - 1/r$ ($\mu \ll 1$, cf. appendix). Note, however, that in this case the absolute numbers of both mutated and nonmutated cells are growing over time. In Figures 4–6, we show the results for scenarios where the relative fitness ranges from $r = 0.8$ to 1.2. Contrary to (Fig. 2), we now choose export probabilities satisfying $q > q_{critical}$ in all cases ($q = 0.1$ for $r = 0.8$ and 1.0 and $q = 0.2$ for $r = 1.2$). Because we use a logarithmic scale

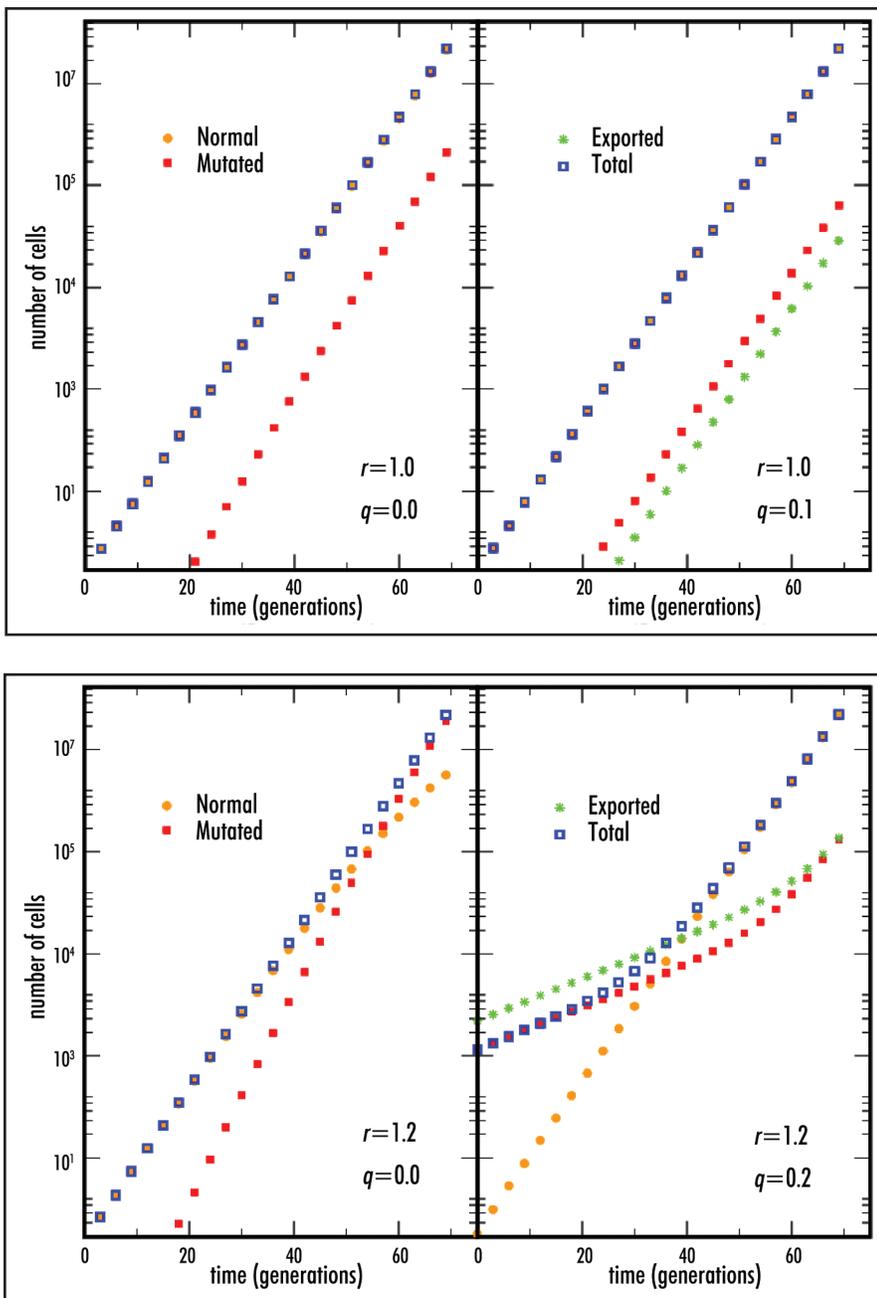


Figure 6. Model B: Dynamics of metastasis formation for a growing tumor, for $r = 1.2$, starting from a single nonmutated cell (mutation rate $\mu = 0.001$). We use same notation and parameter values as in Figure 4. The most interesting dynamics occurs for $r = 1.2$ and $q = 0.2$: Clearly, mutated cells quickly invade most of the tumor. However, because $q > q_{critical}$, normal cells ultimately take over most of the tumor. Note the upward turning of both mutated and exported cells, indicating an approach to the predicted quasi-stationary regime.

for the number of cells, straight lines indicate exponential growth. The results correspond to an average over 1000 simulations carried out for each combination of parameters. Tumor growth is stopped when $N_{threshold} = 10^7$ tumor cells are reached and the plots show the dynamics associated with the last 75 generations. For $r = 0.8$ (Fig. 4), the tumor is mainly composed of nonmutated cells, and an export probability of $q = 0.1$ serves to deplete the tumor of mutated cells. For $r = 1.0$ (Fig. 5), a value of $q = 0.1$ leads to the quasi-stationary regime predicted by the analytical model, which means that the straight lines associated with total and mutated cells are parallel.

Figure 5. Model B: Dynamics of metastasis formation for a growing tumor, for $r = 1.0$, starting from a single nonmutated cell (mutation rate $\mu = 0.001$). We use same notation and parameter values as in Figure 4. For $r = 1.0$, a value of $q = 0.1$ leads to the quasi-stationary regime predicted by the analytical model, which means that the straight lines associated with total and mutated cells are parallel.

An interesting dynamics occurs for $r = 1.2$ and $q = 0.2$ (cf. Fig. 6): clearly, mutated cells quickly invade most of the tumor in the initial phase. As $N_{threshold}$ is reached, the number of mutated cells starts approaching the predicted quasi-stationary regime of $m = 0.025$. Again, for $q > q_{critical}$ the export probability is detrimental to the expansion of metastasis-enabled cells inside the original tumor. Note that, in spite of the similarity in the characterization of the quasi-stationary regimes in both static and growing tumors, the detailed dynamics are very different, as well as the overall number of exported cells. Indeed, growing tumors have a much higher potential of inducing dire consequences in the patient.

DISCUSSION

The development of tumor metastases is a complex biological process and both tumor and host-specific genetic factors play a determining role in their origins.⁴⁰⁻⁴² Acquisition of the ‘metastatic phenotype’ implies that tumor cells must detach from the local tumor extracellular matrix and avoid anoikis, enter into either draining lymphatic channels or veins, and after surviving the trip leave the circulation to establish a niche in an environment that normally does not support their growth.^{40,42} Recently, this process has been shown to require the recruitment of bone marrow derived cells that establish the new niche for tumor cells to develop a metastasis.⁴³ Tumors shed cells in the circulation at a rapid rate but metastases formation is inefficient due to the complex interplay of factors necessary for the establishment of the metastatic niche.^{34,44}

Although carcinogenesis is a multi-step process that requires the interaction of many mutations, isolated mutations activating MYC or RAS⁴⁵ allow cells to develop metastases in experimental models. In addition, inactivation of an ever increasing list of “metastases suppressor genes” (MSG) also facilitates this process.⁴⁶⁻⁴⁸ The rate of tumor growth is related to both the proliferation and death rate of cells and the probability of cells to leave the primary tumor site. The interplay of these parameters determines the phenotypic behavior of tumors and explains the wide heterogeneity that is seen within tumors of the same tissue origin in different patients.

In this report, we studied the intra-tumor evolution of cells that acquire the ability to metastasize due to mutation coupled with a finite probability for mutated cells to be exported.³⁴ In the context of either a stationary tumor population or tumor growth, export probability and relative fitness of the mutated cells both play a determining

role in the evolutionary trajectory of the mutated cell population within the tumor. A key determinant of the outcome is the relative fitness, r , of mutated cells compared to nonmutated tumor cells. Although neutral mutants ($r = 1$) may ultimately dominate the tumor population, fixation is unlikely for mutants with reduced fitness ($r \leq 1$) and at any time, the mutant cells represent a small fraction of the whole population.³⁵ A nonzero export probability ($q > 0$) in the absence of tumor growth decreases even further the small size of the mutated population. This also has a negative impact on the absolute number of cells exported from the tumor since their generation is mainly due to mutation and not expansion within the primary tumor. However, when the tumor grows exponentially, the population of mutated cells will increase despite both a fitness disadvantage and a nonzero export probability. As expected, any mutation that increases the fitness of cells, even in the presence of a nonzero export probability, results in population growth with an increase in absolute cell export. If the export probability is sufficiently high, this process may ultimately prove detrimental for the expansion of mutated cells in the primary tumor. As shown in Figures 4–6, the negative effect of a high export probability can be offset by a higher fitness of the mutants since they will be selected for replication more often. If the probability for initiation of metastases elsewhere is small, then a high q may decrease the net development of metastases unless the primary tumor is growing. In such a scenario, the mutation rate also becomes important and for a given fitness, a higher mutation rate is of benefit to the tumor since it increases the rate of generation of cells with the metastatic phenotype although at the potential cost of accumulating deleterious mutations. Current thinking assumes that most tumors are composed of a homogenous population of cells.⁴⁹ This concept may be true in the presence of mutants that have either neutral fitness or a higher fitness compared to the rest of the tumor since the mutants will ultimately take over the population. However, in the presence of a nonzero export probability, clonal dominance by the mutants need not occur, favoring more heterogeneous tumor cell populations in agreement with other observations.^{10,50} Our model is also compatible with observations from colorectal and urothelial cancer.^{51,52} In both types of tumor, it has been shown that metastases enabled cells are part of a subclone within the primary tumor and support our model whereby cells that acquire metastatic ability can expand within the primary tumor or exit to form metastases.

Our modeling suggests that tumors can be divided into two broad categories: (i) In some tumors, almost all the cells have acquired the metastatic phenotype and are able to leave the primary site and develop metastases elsewhere. These tumors have mutations in genes such as *c-myc* or *ras* that give mutant cells both a proliferative advantage in the primary tumor ($r > 1$) and the ability to form metastases.⁴⁵ (ii) Other tumors only have a small population of cells with metastatic ability. These tumor cells have mutations in *MSG* that do not give the cells a selective advantage within the environment of the primary tumor.⁴⁷ The population of cells with mutations in *MSG* are very sensitive to the export probability since they have a small $q_{critical}$ whereas tumor cells with mutations in genes such as *myc* and *ras* will have a higher $q_{critical}$ due to their selective advantage within the primary tumor itself.

In our previous model, where net cell export was not considered at par with the tumor stochastic dynamics, the presence of a higher fitness would ultimately lead to fixation of the mutant population within the primary tumor.³⁵ However, when mutated cells have a nonzero probability of leaving the tumor, and this feature is included simultaneously with tumor evolutionary dynamics, the trajectory of

the mutated cells need not always lead to fixation, even when they have a higher fitness. Indeed, a high export probability may deplete the primary tumor of cells with the metastatic phenotype and so lower the probability of metastasis formation compared to a tumor with a lower q .

The rich plethora of scenarios emerging from the detailed interplay between r , μ and q in our current model can explain the presence of diffuse metastatic disease in the absence of a firm diagnosis of a primary tumor. Indeed, the primary tumor may be sufficiently small to evade detection with available technologies and at autopsy. In a tumor with a low net intrinsic growth rate but high mutation rate, the probability of the acquisition of the metastatic phenotype can be high. For such a tumor, a high export probability may lead to a small, perhaps undetectable, primary tumor but with many metastases. An open and challenging problem relies on incorporating the stochastic dynamics of metastatic niche formation.

In summary, the dynamic interactions between mutation rate, relative fitness of mutants and export probability all contribute to determine the rate of metastases formation in cancer. For any tumor, there is an optimal combination where the potential for the emergence of metastases is highest. Although a very high export probability may increase the rate of metastases formation originating from a tumor of constant size, such a high rate may be detrimental even for a tumor exhibiting exponential growth, since it will deplete the tumor of cells that otherwise would be selected to divide and contribute to the growth of the tumor. Our model implies that there is a nonlinear relationship between mutation rate, metastatic phenotype, export probability and tumor growth rate. These interactions explain the wide biological heterogeneity seen within tumors even when arising within the same tissue.

References

- Horwich A, Shipley J, Huddart R. Testicular germ-cell cancer. *Lancet* 2006; 367:754-65.
- Wieder R. Insurgent micrometastases: Sleeper cells and harboring the enemy. *J Surg Oncol* 2005; 89:207-10.
- Braun S, Vogl FD, Naume B, Janni W, Osborne MP, Coombes RC, Schlimok G, Diel IJ, Gerber B, Gebauer G, Pierga JY, Marth C, Oruzio D, Wiedswang G, Solomayer EF, Kundt G, Strobl B, Fehm T, Wong GY, Bliss J, Vincent-Salomon A, Pantel K. A pooled analysis of bone marrow micrometastasis in breast cancer. *N Engl J Med* 2005; 353:793-802.
- Keene SA, Demeure MJ. The clinical significance of micrometastases and molecular metastases. *Surgery* 2001; 129:1-5.
- Joensuu H, Kellokumpu-Lehtinen PL, Bono P, Alanko T, Kataja V, Asola R, Utriainen T, Kokko R, Hemminki A, Tarkkanen M, Turpeenniemi-Hujanen T, Jyrkkio S, Flander M, Helle L, Ingalsuo S, Johansson K, Jaaskelainen AS, Pajunen M, Rauhala M, Kaleva-Kerola J, Salminen T, Leinonen M, Elomaa I, Isola J. Adjuvant docetaxel or vinorelbine with or without trastuzumab for breast cancer. *N Engl J Med* 2006; 354:809-20.
- Andre T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, Topham C, Zaninelli M, Clingan P, Bridgewater J, Tabah-Fisch I, de Gramont A. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. *N Engl J Med* 2004; 350:2343-51.
- Winton T, Livingston R, Johnson D, Rigas J, Johnston M, Butts C, Cormier Y, Goss G, Incelet R, Vallieres E, Fry W, Bethune D, Ayoub J, Ding K, Seymour L, Graham B, Tsao MS, Gandara D, Kesler K, Demmy T, Shepherd F. Vinorelbine plus cisplatin vs. observation in resected nonsmall-cell lung cancer. *N Engl J Med* 2005; 352:2589-97.
- Chorost MI, Lee MC, Yeoh CB, Molina M, Ghosh BC. Unknown primary. *J Surg Oncol* 2004; 87:191-203.
- Ghosh L, Dahut W, Kakar S, Posadas EM, Torres CG, Cancel-Santiago R, Ghosh BC. Management of patients with metastatic cancer of unknown primary. *Curr Probl Surg* 2005; 42:12-66.
- Weigelt B, Peterse JL, van 't Veer LJ. Breast cancer metastasis: Markers and models. *Nat Rev Cancer* 2005; 5:591-602.
- Boi S, Amichetti M. Late metastases of cutaneous melanoma: Case report and literature review. *J Am Acad Dermatol* 1991; 24:335-8.
- Demicheli R, Retsky MW, Swartzendruber DE, Bonadonna G. Proposal for a new model of breast cancer metastatic development. *Ann Oncol* 1997; 8:1075-80.
- O'Reilly MS, Holmgren L, Shing Y, Chen C, Rosenthal RA, Moses M, Lane WS, Cao Y, Sage EH, Folkman J. Angiostatin: A novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. *Cell* 1994; 79:315-28.

14. Schirrmacher V. T-cell immunity in the induction and maintenance of a tumour dormant state. *Semin Cancer Biol* 2002; 11:285-95.
15. Kendal WS. Fractal heterogeneity of peripheral blood flow: Implications for hematogenous metastases. *J Surg Oncol* 2000; 74:116-21.
16. Retsky MW, Demicheli R, Swartzendruber DE, Bame PD, Wardwell RH, Bonadonna G, Speer JF, Valagussa P. Computer simulation of a breast cancer metastasis model. *Breast Cancer Res Treat* 1997; 45:193-202.
17. Kiricuta CI, Tausch J. A mathematical model of axillary lymph node involvement based on 1446 complete axillary dissections in patients with breast carcinoma. *Cancer* 1992; 69:2496-501.
18. Delsanto PP, Romano A, Scalerandi M, Pescarmona GP. Analysis of a "phase transition" from tumor growth to latency. *Phys Rev E Stat Phys Plasmas Fluids Relat Interdiscip Topics* 2000; 62:2547-54.
19. Yorke ED, Fuks Z, Norton L, Whitmore W, Ling CC. Modeling the development of metastases from primary and locally recurrent tumors: Comparison with a clinical data base for prostatic cancer. *Cancer Res* 1993; 53:2987-93.
20. Moolgavkar SH, Knudson Jr AG. Mutation and cancer: A model for human carcinogenesis. *J Natl Cancer Inst* 1981; 66:1037-52.
21. Sherratt JA. Mathematical modelling of cancer invasion and metastasis: An interdisciplinary workshop held at the University of Warwick, Coventry, UK. 11-13 September 1996. *Clin Exp Metastasis* 1997; 15:63-74.
22. Luebeck EG, Moolgavkar SH. Multistage carcinogenesis and the incidence of colorectal cancer. *Proc Natl Acad Sci USA* 2002; 99:15095-100.
23. Nowak MA, Komarova NL, Sengupta A, Jallepalli PV, Shih Ie M, Vogelstein B, Lengauer C. The role of chromosomal instability in tumor initiation. *Proc Natl Acad Sci USA* 2002; 99:16226-31.
24. Frank SA, Iwasa Y, Nowak MA. Patterns of cell division and the risk of cancer. *Genetics* 2003; 163:1527-32.
25. Gatenby RA, Maini PK. Mathematical oncology: Cancer summed up. *Nature* 2003; 421:321.
26. Little MP, Wright EG. A stochastic carcinogenesis model incorporating genomic instability fitted to colon cancer data. *Math Biosci* 2003; 183:111-34.
27. Michor F, Frank SA, May RM, Iwasa Y, Nowak MA. Somatic selection for and against cancer. *J Theor Biol* 2003; 225:377-82.
28. Nowak MA, Michor F, Iwasa Y. The linear process of somatic evolution. *Proc Natl Acad Sci USA* 2003; 100:14966-9.
29. Michor F, Nowak MA, Frank SA, Iwasa Y. Stochastic elimination of cancer cells. *Proc Biol Sci* 2003; 270:2017-24.
30. Nowak MA, Michor F, Komarova NL, Iwasa Y. Evolutionary dynamics of tumor suppressor gene inactivation. *Proc Natl Acad Sci USA* 2004; 101:10635-8.
31. Michor F, Hughes TP, Iwasa Y, Branford S, Shah NP, Sawyers CL, Nowak MA. Dynamics of chronic myeloid leukaemia. *Nature* 2005; 435:1267-70.
32. Komarova NL, Wodarz D. Drug resistance in cancer: Principles of emergence and prevention. *Proc Natl Acad Sci USA* 2005; 102:9714-9.
33. Hill RP, Chambers AF, Ling V, Harris JF. Dynamic heterogeneity: Rapid generation of metastatic variants in mouse *B16* melanoma cells. *Science* 1984; 224:998-1001.
34. Wyckoff JB, Jones JG, Condeelis JS, Segall JE. A critical step in metastasis: In vivo analysis of intravasation at the primary tumor. *Cancer Res* 2000; 60:2504-11.
35. Michor F, Nowak MA, Iwasa Y. Stochastic dynamics of metastasis formation. *J Theor Biol* 2006; 240:521-30.
36. Michor F, Iwasa Y. Dynamics of metastasis suppressor gene inactivation. *J Theor Biol* 2006; 241:676-89.
37. Kendal WS. The role of multiple somatic point mutations in metastatic progression. *Math Biosci* 1992; 108:81-8.
38. Michor F. Chromosomal instability and human cancer. *Philos Trans R Soc Lond B Biol Sci* 2005; 360:631-5.
39. Poste G, Doll J, Hart IR, Fidler IJ. In vitro selection of murine B16 melanoma variants with enhanced tissue-invasive properties. *Cancer Res* 1980; 40:1636-44.
40. Weiss L. Metastasis of cancer: A conceptual history from antiquity to the 1990s. *Cancer Metastasis Rev* 2000; 19:1-XI, 193-383.
41. Hunter K. Host genetics influence tumour metastasis. *Nat Rev Cancer* 2006; 6:141-6.
42. Steeg PS. Tumor metastasis: Mechanistic insights and clinical challenges. *Nature Medicine* 2006; 12:895-904.
43. Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, MacDonald DD, Jin DK, Shido K, Kerns SA, Zhu Z, Hicklin D, Wu Y, Port JL, Altorki N, Port ER, Ruggero D, Shmelkov SV, Jensen KK, Rafii S, Lyden D. VEGFR1-positive haematopoietic bone marrow progenitors initiate the premetastatic niche. *Nature* 2005; 438:820-7.
44. Luzzi KJ, MacDonald IC, Schmidt EE, Kerkvliet N, Morris VL, Chambers AF, Groom AC. Multistep nature of metastatic inefficiency: Dormancy of solitary cells after successful extravasation and limited survival of early micrometastases. *Am J Pathol* 1998; 153:865-73.
45. Wylie AH, Rose KA, Morris RG, Steel CM, Foster E, Spandidos DA. Rodent fibroblast tumours expressing human *myc* and *ras* genes: Growth, metastasis and endogenous oncogene expression. *Br J Cancer* 1987; 56:251-9.
46. Berger JC, Vander Griend D, Stadler WM, Rinker-Schaeffer C. Metastasis suppressor genes: Signal transduction, cross-talk and the potential for modulating the behavior of metastatic cells. *Anticancer Drugs* 2004; 15:559-68.
47. Steeg PS. Perspectives on classic article: Metastasis suppressor genes. *J Natl Cancer Inst* 2004; 96:E4.
48. Shevde LA, Welch DR. Metastasis suppressor pathways-an evolving paradigm. *Cancer Lett* 2003; 198:1-20.
49. Weigelt B, Hu Z, He X, Lvasy C, Carey LA, Ewend MG, Glas AM, Perou CM, Van't Veer LJ. Molecular portraits and 70-gene prognosis signature are preserved throughout the metastatic process of breast cancer. *Cancer Res* 2005; 65:9155-8.
50. Kang Y, Siegel PM, Shu W, Drobniak M, Kakonen SM, Cordon-Cardo C, Guise TA, Massague J. A multigenic program mediating breast cancer metastasis to bone. *Cancer Cell* 2003; 3:537-49.
51. Jones TD, Carr MD, Eble JN, Wang M, Lopez-Beltran A, Cheng L. Clonal origin of lymph node metastases in bladder carcinoma. *Cancer* 2005; 104:1901-10.
52. Thebo JS, Senagore AJ, Krygier S, Luchtfeld MA. *K-ras* mutational analysis of polyclonal colorectal cancers identifies uniclonal circulating tumor cells. *Am Surg* 2001; 67:802-5.
53. Antal T, Scheuring I. Fixation of strategies for an evolutionary game in finite populations. *Bull Math Biol* 2006; 10, (1007/s11538-006-9061-4).

APPENDIX

Model A. Let us consider a constant population of N tumor cells, in which $M \leq N$ cells exhibit the metastatic phenotype. We describe the state of the system in terms of the number of mutated cells, M . According to the model, the probability π_M^+ that M increases can be written as

$$\pi_M^+ = p_M(1-q)\frac{N-M}{N} + (1-p_M)\mu\frac{N-M}{N}$$

where

$$p_M = \frac{rM}{rM + N - M}$$

is the probability to select a mutated cell proportional to fitness. Probability π_M^+ is the sum of two terms: the first term corresponds to the probability of selecting a mutated cell, times the probability that this cell is *not* exported ($1 - q$), times the probability that the cell chosen for death is a normal cell. The second term corresponds to the probability that a normal cell is selected for replication, times the probability it mutates, times the probability that (again) a normal cell is selected for death. These are the only two processes which lead to an increase from M to $M + 1$ mutated cells.

The probability π_M^- that the number of mutated cell decreases by one is given by

$$\pi_M^- = (1-p_M)(1-\mu)\frac{M}{N}$$

Indeed, this is simply the product of the probability that a normal cell is chosen for replication times the probability that it does *not* mutate, times the probability that a mutated cell is chosen for death.

The state in which all cells are finally mutated is the only absorbing state of this Markov process. However, for large N , it is possible that the system gets trapped into a quasi-stationary state (which is different from the absorbing state), in the sense that the system may spend an extremely long time in the vicinity of this state (in fact, it can be shown that this time increases roughly exponentially with the population size).⁵³ In other words, the system may behave as if it has reached a stationary state. This state is characterized by both probabilities π_M^+ and π_M^- being equal. Whenever $\pi_M^+ = \pi_M^-$ we obtain the fraction of mutants $m = M / N$ in the population characterizing this quasi-stationary regime

$$m = \begin{cases} 1 & \text{for } q < q_{critical} \\ \frac{\mu}{1-r(1-q)} & \text{for } q > q_{critical} \end{cases}$$

where $q_{critical} = 1 - (1 - \mu)/r$. From these expressions we can also calculate another threshold value of the export probability, $q_{1/2} = 1 - (1 - 2\mu)/r$ above which the number of mutated cells stops exceeding that of normal cells.

We can also determine the rate of export of mutated cells from the system. Initially, there is a (short) transient state until the population reaches the quasi-stationary regime. Subsequently, the number of mutated cells exported, N_q , is proportional to time: $N_q = NR_M t$, where R_M is the export rate of mutated cells and t is measured in generations. Since the probability that a mutant leaves the system in one elementary time step is qp_M , from the quasi stationary density m we arrive at the following expression for R_M

$$R_M = \begin{cases} q & \text{for } q < q_{critical} \\ \frac{qr\mu}{1 - \mu - r(1 - q - \mu)} & \text{for } q > q_{critical} \end{cases}$$

The rate is maximal at $q = q_{critical}$. The behavior of both m and R_M is shown in (Fig. 3).

Model B. Model B assumes tumor growth. Birth events occur with a probability b whereas death events occur with a probability d , and when $b > d$ the tumor grows. Starting from a single cell, the stochastic nature of the dynamics may lead to extinction of the tumor. Discarding these events and when the total number of cells N (now a function of time) becomes sufficiently large, we may approximate the stochastic dynamics by deterministic dynamics. The continuous equations determining the dynamics of the expected values of normal and mutated cells are given by

$$\frac{dM}{dt} = Nb[p_M(1 - q) + (1 - p_M)\mu] - dM$$

$$\frac{dN}{dt} = N(b - d - bqp_M)$$

The rate of change is the balance between a growing term proportional to b and a decreasing term proportional to d . The growing term has exactly the same form as model A except for the $(N - M) / N$ term associated (in model A) with the death of normal cells. The decreasing term reflects the death of mutated cells, which is proportional (with uniform probability) to the fraction of mutated cells in the population at time t .

The second equation similarly governs the net growth of the population size, N , as a balance between growth of normal cells (proportional to b), the decrease due to cell death (proportional to d) and the decrease due to the export of mutated cells, once selected for replication during a birth event. The N factor in both equations renders the time in generations.

The equation governing the dynamics of the density of mutated cells $m = M / N$ as a function of time is given by

$$\frac{dm}{dt} = \frac{1}{N} \left(\frac{dM}{dt} - m \frac{dN}{dt} \right) = b(1 - m) \frac{m(r - 1 - qr) + \mu}{rm + (1 - m)}$$

Note that the equation above does not depend on the death rate, as death events do not discriminate between normal and mutant cells. The equation above shows that, if mutants invade the entire tumor ($m = 1$), then the absorbing state has been reached in which all tumor cells are metastasis enabled. Also when $m = 0$, the density of mutants will grow: $dm / dt = b\mu / N > 0$. Similar to the case discussed in model A, there is a stable fixed point m at which $dm / dt = 0$, that

is, the density will remain constant (at this level of approximation). This point is precisely given by the same equation for m derived before, for a critical export probability $q_{critical}$ satisfying also the same equation as in model A. Note however, that unlike in model A, the population is growing in model B: At the fixed point the ratio M / N remains (approximately) constant, although both numbers grow in absolute value.