A microenvironmental model of carcinogenesis

Robert A. Gatenby and Robert J. Gillies

Abstract | We propose that carcinogenesis requires tumour populations to surmount six distinct microenvironmental proliferation barriers that arise in the adaptive landscapes of normal and premalignant populations growing from epithelial surfaces. Somatic evolution of invasive cancer can then be viewed as a sequence of phenotypical adaptations to these barriers. The genotypical and phenotypical heterogeneity of cancer populations is explained by an equivalence principle in which multiple strategies can successfully adapt to the same barrier. This model provides a theoretical framework in which the diverse cancer genotypes and phenotypes can be understood according to their roles as adaptive strategies to overcome specific microenvironmental growth constraints.

Carcinogenesis is a complex, multistep, multipath process often described as ‘somatic evolution’. Models of carcinogenesis are typically based on the Darwinian principle that evolution requires genetic and/or epigenetic changes that generate new phenotypes. For example, Fearon and Vogelstein depict a sequence of specific heritable events that cause corresponding changes in tumour size and morphology during colorectal carcinogenesis. Hanahan and Weinberg emphasized the final evolutionary outcomes by distilling the properties of transformed cells to six phenotypical hallmarks that are necessary for an invasive cancer.

These approaches correspond to empirical observations but do not necessarily offer insight into the evolutionary dynamics that determined the nature and sequence of the observed phenotypical changes. In the words of the current cell-centric models, they do not typically address why these specific phenotypical and genotypical changes are necessary for carcinogenesis or why they occur in a sequence such as that depicted in the Fearon–Vogelstein diagram. This reflects the limits of models that are focused on just one component of the evolutionary process — adaptive changes observed in evolving populations. In fact, Darwinian dynamics consist of the interactions of individual phenotypes with environmental selection forces that govern fitness and, therefore, proliferation. In the adaptive landscapes governing somatic evolution, microenvironmental barriers to proliferation represent crucial selection forces that control the proliferative capacity of any new phenotype that is generated by genetic or epigenetic events. Thus, the consistent phenotypical changes that emerge during carcinogenesis must always represent successful adaptations to these microenvironmental selection forces. Furthermore, these selection pressures are not static but will dynamically change as a result of tumour population growth and evolution.

Here, we propose a theoretical model of carcinogenesis based on the microenvironmental proliferative barriers that will arise as tumour populations proliferate on basement membranes. By acting as growth inhibitors, the barriers determine the fitness and, therefore, clonal growth of each new population generated by random mutations or epigenesis. As such, they select for specific adaptive cellular properties during the different stages of carcinogenesis and ultimately determine the phenotypical properties of invasive cancers. Through this model we hope to provide insight into the evolutionary forces that produce the specific phenotypical and genotypical changes that are hallmarks of malignant populations and a rationale for the sequence with which these changes may occur during carcinogenesis.

Adaptive landscapes in carcinogenesis

We propose that the microenvironmental selection forces during early carcinogenesis are determined by the anatomy and physiology of epithelial surfaces, and the phenotypical properties of the normal and tumour cells present. As shown in FIG. 1, epithelial cells are separated from the underlying mesenchyma, including blood vessels, by an intact basement membrane. Substrates, metabolites and signalling molecules exchanged between epithelial cells and the mesenchyma (that is, fibroblasts, extracellular matrix, immune cells and blood vessels) must diffuse across this basement membrane. As shown in FIG. 1a,b, the average diffusion length between proliferating cells and the parenchyma

DATABASES

CDKN1A | CDKN1B | CDK4 | CDK6 | CCND1 | CCNE1 | CDH1 | CTNNB1 | E2F1 | E2F2 | E2F3 | E2F4 | E2F5 | E2F6 | ERBB2 | ERBB3 | ERBB4 | FGFR1 | FGFR2 | FGFR3 | FGFR4 | FLI1 | FOS | FOXA1 | JAK2 | JUN | Kras | LRPS | MAP2K1 | MET | MNT | NCOA1 | NCOA2 | NCOA3 | NCI | NEUROD1 | NF1 | NF2 | NF3 | NF4 | NF5 | NF6 | NF7 | NF9 | NFE2L2 | P27 | PDGFRA | PDGFB | PIK3CA | PIK3R1 | PIK3R2 | PIK3R3 | PKHD1 | PTEN | RAF1 | RAF2 | RB1 | RBL1 | RBL2 | RNF213 | RBX1 | RRM2 | RRM2L | TP53 | TSC1 | TSC2 | TRAF2 | TRAF3 | TRAF5 | TRAF6 | TXNIP | WT1 | ZBTB17 | ZEB1 | ZEB2

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varies during carcinogenesis. In normal epithelium (FIG. 1a), a short diffusion distance permits physiological levels of growth factors, substrate and metabolites. However, the diffusion length increases as the hyper-proliferation following initial mutations carries cells away from the basement membrane. Because tumour cells remove growth factors and substrates and produce metabolites, diffusion–reaction kinetics will create significant gradients along the axis perpendicular to the membrane. This forms the adaptive landscape of FIG. 1b, in which cells proliferating into the lumen experience decreased local concentrations of vascular or mesenchymal-derived growth factors and vital substrates such as glucose and oxygen.

We propose that a different and profoundly important adaptive landscape emerges during microinvasion (FIG. 1c). This is a crucial period in the evolution of an invasive cancer because it involves, for the first time, direct contact between the epithelial tumour cells and the surrounding stroma. In this novel adaptive landscape, the Darwinian dynamics of pre-malignant tumours will persist as the transformed epithelial cells continue to compete with each other and with the local mesenchymal cells for space and resources. However, some degree of cooperation is also necessary because unrestricted tumour growth requires stromal support for angiogenesis and formation of the extracellular matrix.

Thus, delivery of substrate and growth factors requires vascularization of the tumour mass, and evolution of a clinically significant invasive cancer from a focus of microinvasion requires not simply growth of the cancer population but rather an active collaboration of malignant epithelial cells and normal mesenchymal cells.

**Barriers to carcinogenesis**

The dynamics of *in vivo* cellular proliferation on a basement membrane have been described through the equation in BOX 1. We have explored this model extensively in prior publications and found that it provides important insights into the evolutionary dynamics of carcinogenesis.
particular in analysing the role of substrate limitation in carcinogenesis. Here, we wish to propose a general theoretical model of carcinogenesis based on the barriers to proliferation that develop as tumours grow and evolve on epithelial surfaces. We demonstrate that these barriers also correspond to crucial parameters within this mathematical model of in vivo tissue growth. Although many insights have been obtained from these quantitative methods, our goal is to present this work to a general scientific audience and refer the mathematically inclined reader to prior publications.

At any given time, the total population and, therefore, size and morphology of the corresponding malignant or premalignant lesion is a summation of all extant populations. The right side of the equation defines the barriers to cellular proliferation. All parameters with the subscript \( i \) can be affected by the phenotype of the population.

\[
\frac{dx_i}{dt} = x_i \left( b_i(y,r) - d_i(t) \right) \left( 1 - \frac{\sum x_j K_j}{K_i} \right) \left( S_i(y,r) - m_i \right)
\]

\( x_i \) represents the number of cells with phenotype \( i \). At any given time, the total population and, therefore, size and morphology of the corresponding malignant or premalignant lesion is \( \sum x_i \). All parameters with subscript \( i \) can be altered by the phenotypic properties of the cell. For example, the proliferation rate, \( b(y,r) \), is influenced by local concentrations of growth factors which vary by spatial location \( y \) (the distance from the basement membrane or nearest blood vessel) and blood flow \( r \). However, the phenotype may, through autocrine production of growth factors or upregulation of transduction pathways, become relatively independent of these factors. \( b(y,r) - d_i(t) \) is the net proliferation rate of the population (the death rate subtracted from the proliferation rate).

\( d_i(t) \) is the death rate. This is a function of time \( t \) because of senescence (that is, telomere shortening), so that older populations will generally have a greater death rate.

\( b(y,r) \) is the proliferation rate. This is a measure of growth promoters in the microenvironment. As noted in the text, the proliferation rate \( b(y,r) \) is influenced by local concentrations of growth factors, which are typically derived from the blood or mesenchyma. In premalignant tumours, this concentration is spatially dependent because of the diffusion distance of the cell from the basement membrane \( y \). In invasive cancer this will be dependent on blood flow \( r \) as well as the diffusion distance from the nearest vessel.

\[
[1 - \sum x_i K_i / K_i] \text{ is a Lotka–Volterra term defining the cellular interactions where } K_i \text{ is the carrying capacity, determined by the interaction of each cell with other cells and the basement membrane. For example, the number of normal epithelial cells lining a duct is maintained by limits on crowding (contact inhibition) and the requirement for continuous contact with the basement membrane.}
\]

\( \alpha_i \) is the proliferative consequence for members of one phenotype due to the presence of other members of the same population \( \alpha_i \) or members of another population \( \alpha_j \). This includes the effect of consuming space and resources as well as growth promotion or inhibition through the production of positive and negative growth factors.

\( S_i(y,r) - m_i \) is the substrate availability. Assume that each cell has a basal substrate requirement \( m_i \) to maintain viability. Substrate uptake, \( S_i(y,r) \), must be greater than \( m_i \) to allow proliferation. In premalignant lesions, substrate and metabolite concentrations are spatially dependent \( y \) because the diffusion–reaction kinetics produce a decrease in substrate concentration with distance from the basement membrane. In invasive tumours, substrate delivery is dependent on blood flow \( r \) as well as the distance of the tumour cells from the nearest blood vessel.

FIGURE 1a demonstrates that normal epithelium possesses physiological amounts of substrate and growth factors. The lumen provides a large potential space into which the epithelial cells may proliferate. However, growth into this empty volume is prevented by anoikis (detachment-induced apoptosis) and proliferation of cells along the basement membrane is limited by contact inhibition generated by neighbouring cells. These barriers are represented by the \( \alpha_i \) and \( K_i \) terms in the equation. As these are the dominant proliferation barriers in normal epithelium, the first genetic changes in carcinogenesis must produce a phenotypical adaptation to anoikis or contact inhibition.

Thus, genotypical changes in early carcinogenesis must involve pathways that control these proliferative barriers (FIG. 2). This prediction is consistent with experimental observations that pathways that affect anoikis and/or contact inhibition are altered in early carcinogenesis. Genetic changes in phosphatidylinositol 3-kinase11, E-cadherin (encoded by CDH1)15, Ras16, p63 (REF. 14), adenomatosis polyposis coli (APC), \( \beta \)-catenin (encoded by CTNNB1)15 and oestrogen receptors have been suggested to have roles in these adaptations18. The number and diversity of these control pathways exemplifies a governing principle of our model — that of functional equivalence. That is, each parameter in the equation that constitutes a barrier to carcinogenesis is a general phenomenological term affected by multiple biological pathways, each typically consisting of many signalling biomolecules that often exhibit cross-talk and interdependence. The stochastic nature of genetic mutations will result in random alteration of multiple different gene products that could potentially serve as strategies to overcome any given barrier. The functional equivalence principle states that various mutations and epigenetic changes can alter the value of parameters in the equation and serve as adaptations to growth barriers. These changes, therefore, are both mathematically and biologically equivalent. This principle allows a large number of genotypes to be tumorigenic because alterations in many different pathways or components of pathways may produce phenotypes that are adapted to a given proliferation barrier. The principle of functional equivalence accounts for the observed genetic differences between tumours. Furthermore, multiple genetic clones may adapt to the same proliferation barrier within the same tumour in different regions or at different times. This will result in intratumoral genetic diversity. However, all invasive cancer populations must possess at least one strategy that overcomes each barrier.
decrease the \( b \) term, halting proliferation. This new barrier is overcome through various strategies, including autocrine or paracrine production of growth factors\(^7\), increased expression of membrane receptors\(^8\) or upregulation of elements within signal-transduction pathways\(^9\). The equivalence principle is again evident in that multiple phenotypical strategies may overcome the same proliferation barrier.

This stage also introduces the principle of multibarrier effects. For example, mutations in Ras can affect both proliferation and senescence\(^10\). Similarly, apoptosis can be a common pathway for the cell death caused by anoikis, hypoxia and acidosis. If the tumour cells become resistant to anoikis through evasion of apoptosis in the early carcinogenesis, this property may also confer resistance to hypoxia and acidosis during the later stages. Thus, in some cases, an adaptive strategy to one barrier may increase, lower or eliminate subsequent barriers.

As the tumour cells proliferate, they will eventually be constrained by senescence at the Hayflick limit\(^11\), a result of telomere shortening (50–100 bp per cell division) during mitosis. After a number of generations, the loss of telomere protection of chromosomal ends leads to end-to-end chromosomal fusions that trigger apoptosis (senescence). This barrier is placed arbitrarily in FIG. 2 but could occur at a number of different points during carcinogenesis. Regardless of the timing, it will impose a limit to tumour growth. Subsequent evolution requires an adaptation that confers immortality. Strategies include upregulation of telomerase but, consistent with the multibarrier principle, may also involve changes in oncogenes such as Ras\(^12\).

We have previously pointed out that the increased diffusion distances in the adaptive landscape of FIG. 1b will result in regional hypoxia\(^6\). This produces an additional barrier to proliferation as ATP production from aerobic glucose metabolism falls below maintenance requirement\(^13\), leading to an upregulation of anaerobic ATP production through glycolysis. We have also pointed out that this phenotype can become fixed through the stabilization of hypoxia-inducible factor 1 (HIF) and/or upregulation of phospho-MYC (F. Robey et al., unpublished results) so that elevated glycolysis is maintained, even under aerobic conditions. This metabolism of glucose to lactic acid in the presence of oxygen despite its inefficiency in ATP yield and increased production of acid (the Warburg effect) has been proposed as an additional hallmark of cancer\(^14\).

The glycolytic adaptation results in increased acid production and regional acidosis, which produces a new growth barrier by inducing apoptosis via p53-dependent pathways through increased caspase activity\(^15\). In the equation this increases the value of the interference term (\( c \)) for the glycolytic population. Adaptations to limit acid-mediated toxicity can include equivalent strategies, such as increased activity of Na+/H+ exchangers, p53 mutations and the interruption of caspase pathways. We propose that these adaptations confer a significant growth advantage because they allow the cancer population to alter its environment (through the production of increased acid) in a way that is toxic to other populations but not to itself\(^16,10\). In addition, the acidic extracellular pH leads to increased motility and invasion\(^17\), which will promote breaching of the basement membrane and movement of tumour cells into the adjacent normal tissue\(^18,19\). This promotes adoption of the invasive phenotype as discussed below.

Our theoretical model explicitly predicts that these phenotypical changes should be seen in the later stages of carcinogenesis. This is consistent with clinical observations that increased glucose uptake or GLUT1 (also known as SLC2A1) expression is seen in the late stages of in situ cancers in the oesophagus, colon and breast\(^20,28,30\).

Finally, as the invasive cancer grows it creates a new adaptive landscape in which tumour cells are in direct contact with mesenchymal cells (FIG. 2). In this microenvironment, growth is limited primarily by vascular delivery of substrate (ischaemia) as noted by Folkman\(^31\). This barrier requires angiogenesis through, for example, upregulation of vascular endothelial growth factor (VEGF). Adaptation to this final barrier (that is,
after the basement membrane is breached) is observed experimentally in the transition from limited growth in an avascular state to rapid tumour expansion following acquisition of the angiogenic phenotype. Consistent with the multibarrier principle, this may be affected by prior adaptive strategies such as MYC and p53 mutations.

Even in well vascularized tumours, cellular proliferation alone will eventually be limited by volume constraints imposed by the extracellular matrix and normal tissue structures. This is undoubtedly governed by tissue characteristics so that, for example, tumour growth from proliferation alone may be greater in relatively acellular, pliable tissue such as fat than in the kidney or liver. In other words, non-invasive tumour growth will eventually produce only self-limited, benign lesions, and the unrestricted proliferation that is characteristic of invasive cancers requires additional adaptations to promote invasive growth. These include increased tumour cell motility and degradation of the extracellular matrix. Consistent with the equivalence principle, these adaptations can be accomplished through a number of phenotypical strategies, including upregulation of production and release of extracellular matrix-degrading proteolytic enzymes. In addition, as another example of the multibarrier effect, invasion might also be promoted by upregulation of glycolysis and microenvironmental acidification.

We note that the evolutionary pressures for the invasive phenotype do not emerge directly from the equation. However, this adaptation must be consistent with the fundamental principle of Darwinian dynamics — individual properties evolve only if they increase fitness and, therefore, proliferation. Interestingly, increased motility requires significant expenditure of energy and substrates, reducing their availability for proliferation. So, in the absence of some proliferative reward, invasiveness will actually tend to reduce fitness. We propose that an evolutionary advantage for increased motility occurs only if it permits cells to move from the tumour adaptive landscape in which growth is restricted by limited substrates and competition with other cancer cells to adjacent normal tissue in which substrates are relatively abundant and competing tumour populations are absent. In other words, the energy and substrate cost of the invasive phenotype is rewarded because mobile tumour cells are able to leave a region in which their fitness is relatively low and enter the relatively idyllic adaptive landscape of adjacent normal tissue, in which their fitness is high, allowing them to proliferate rapidly. This self-perpetuating cycle of cellular movement followed by proliferation promotes evolution of the invasive phenotype and promotes continuous influx of cancer cells into normal tissue, including blood and lymphatic vessels. This yields the most lethal of cancer hallmarks — tissue invasion and metastases.

**Conclusion**

We propose a theoretical model of carcinogenesis that identifies six microenvironmental barriers to somatic evolution of malignant phenotypes and corresponding principles that govern phenotypical adaptation to these constraints (FIG. 2). The barriers represent key parameters in the state equations describing in vivo adaptive landscapes of cell growth on epithelial surfaces. These parameters are closely linked to the anatomy and physiology of basement membranes, which separate supporting mesenchyma from normal and premalignant epithelial populations, and will change substantially as tumour cells proliferate and evolve. An invasive cancer emerges only after all of these barriers are overcome. Somatic evolution is, thus, the sequence of cellular adaptations to these changing barriers.

There are several general principles of carcinogenesis that emerge from this analysis. First, microenvironmental dominance — the nature and sequence of heritable changes during carcinogenesis are determined by the specific microenvironmental properties that prevent proliferation within changing adaptive landscapes. Second, phenotypical necessity — phenotypical properties observable in cancer populations do not arise by accident or as epiphenomena. Rather they represent adaptive strategies and will always confer a proliferative advantage within the microenvironment of that tumour. Third, the equivalence principle — the environment selects for phenotypes, not genotypes, and multiple different mutations or epigenetic changes may produce similar phenotypes. The same adaptive advantage may be gained through multiple different phenotypical changes or strategies. Thus, a number of genetic and phenotypical changes may serve as adaptations to the same proliferation barrier. Fourth, gradualism — a cancer is not observed until all tissue barriers to proliferation are overcome. Although a number of different phenotypical strategies may serve as adaptations to the same barrier, all cancers must exhibit at least one adaptation to every barrier. Finally, the multibarrier principle — adaptation to one barrier may decrease or increase the height of subsequent barriers.

The model demonstrates that all invasive epithelial cancer populations must possess traits that overcome all proliferation barriers and that these adaptations generate the hallmarks of the malignant phenotype. Through the equivalence and multibarrier principles, it also accommodates the diversity and inconsistency of genetic properties in cancer populations that are demonstrated by the remarkable genotypical, epigenetic and transcriptomic heterogeneity within cancers that have presumably evolved through common barriers. This approach allows the disparate observed phenotypical changes in cancer populations to be categorized through their role in overcoming specific proliferation barriers.

Robert A. Gatenby and Robert J. Gillies are at the Department of Radiology, University of Arizona, 1501 N Campbell Avenue, Tucson, Arizona 85724, USA.

Robert A. Gatenby is also at the Department of Applied Mathematics, University of Arizona, 1041 East Lowell Street, Tucson, Arizona 85721, USA.

Robert J. Gillies is also at the Department of Biochemistry & Molecular Biophysics, University of Arizona, 617 N Santa Rita Avenue, Tucson, Arizona 85721, USA.

Correspondence to R.A.G.

e-mail: rga@radiology.arizona.edu

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