Evolutionary Theory of Cancer

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As Theodosius Dobzhansky famously noted in 1973, "Nothing in biology makes sense except in the light of evolution," and cancer is no exception to this rule. Our understanding of cancer initiation, progression, treatment, and resistance has advanced considerably by regarding cancer as the product of evolutionary processes. Here we review the literature of mathematical models of cancer evolution and provide a synthesis and discussion of the field.

Key words: **evolution; cancer; mathematical modeling**

Introduction

The use of mathematics in the study of medical conditions has a long history. The earliest work dates back to the 8th century, when Al-Kindi attempted to apply mathematics to pharmacological questions such as describing the strength of drugs (Prioreschi 2002). In the 18th century, Bernoulli analyzed the morbidity and mortality of smallpox and demonstrated the efficacy of vaccination (Bernoulli & Blower 2004). The interaction between mathematics and medicine has proven beneficial for understanding the underlying biology and for designing treatments and diagnosing diseases. Due to its clinical importance, cancer has been of particular interest to theoretical investigators. Since the early 1940s (Charles & Luce-Clausen 1942), mathematical approaches have been developed to explain regularities seen in incidence data (Armitage & Doll 1957; Fisher 1958; Knudson 2001), hereditary predisposition to disease (Knudson 1986; Frank 2007), cancer progression (Tomlinson et al. 1996; Desper et al. 1999; Michor et al. 2006c), and response to treatment (Goldie & Coldman 1983; Komarova & Wodarz 2005; Michor et al. 2006b) (Fig. 1).

Cancer results from evolutionary processes occurring within the body (Nowell 1976). Since evolution describes the temporal changes of a population of individuals due to variation and selection, the concept is highly relevant to neoplasia. Tumors can be viewed from an evolutionary standpoint as collections of cells that accumulate genetic and epigenetic changes, which are then subjected to the selection pressures within a tissue. These normally heritable variations can lead to adaptations of the cells such as induction of angiogenesis or evasion of the immune system. Beneficial heritable changes can cause rapid expansion of the mutant clone since they enable their carriers to outcompete cells that have not accumulated similar improvements. Mutations advantageous to the cancer cell are normally detrimental to the organism, ultimately causing death of both the patient and the tumor. Therefore, neoplastic processes serve as an example for selection acting on different hierarchical levels (Buss 1987): clonal evolution generally selects for increased proliferation, survival, and evolvability on the cellular level and leads to progression, invasion, and resistance; the latter effects are selected against on the level of multicellular organisms.

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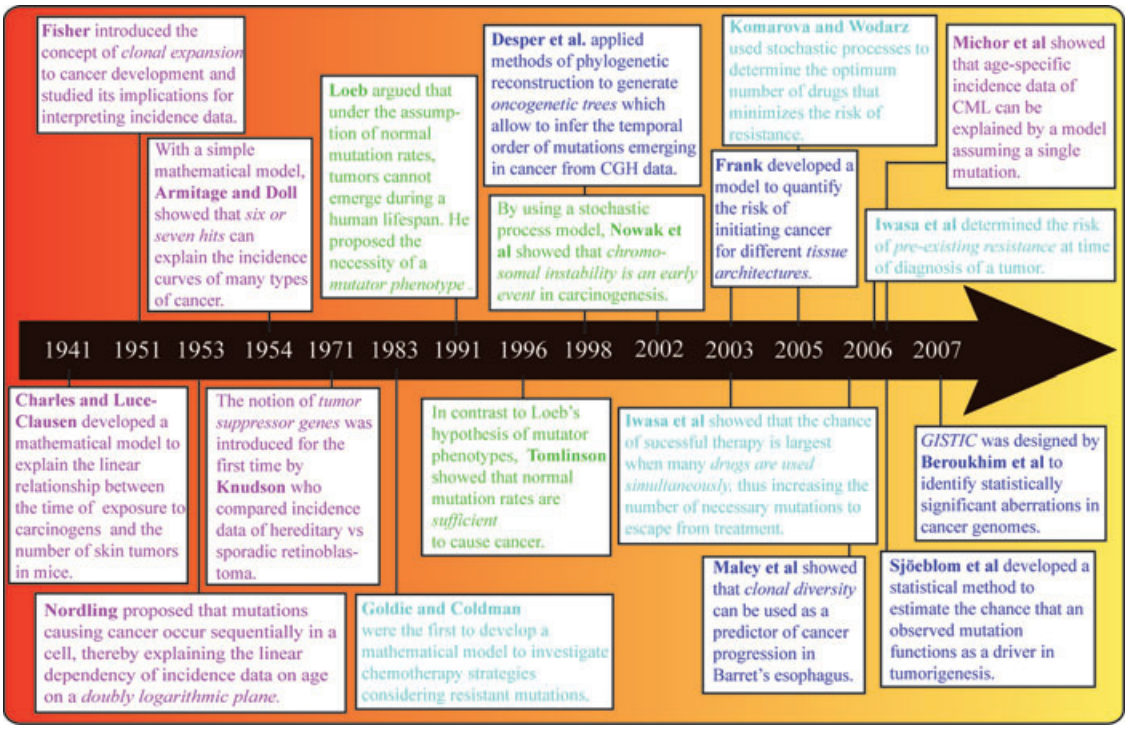


Figure 1. Contributions to an evolutionary theory of cancer. We show examples of theoretical approaches to cancer initiation (black outline), genetic instabilities (dark gray), progression (gray), and resistance (light gray). Online version: cancer initiation (purple), genetic instabilities (green), progression (dark blue), and resistance (light blue).

The investigation of cancer evolution requires mechanistic, quantitative models that incorporate realistic properties of biological systems such as stochasticity and nonlinearity. As the outcomes of such interactions cannot be determined by verbal reasoning alone, they must be computed from general integrative models of carcinogenesis (Gatenby & Maini 2003; Michor et al. 2004a; Merlo et al. 2006). Theoretical approaches to tumorigenesis have led to considerable insights into the natural history of the disease and have begun to transform cancer research into a rational and predictive science. In this review we present a historical and topical view of mathematical models of cancer evolution. We center our attention on several areas of interest in the analysis of cancer—initiation, progression, genomic instabilities, differentiation and heterogeneity, and drug resistance—and provide a synthesis of the field.

Cancer Initiation

An understanding of the mechanisms of cancer initiation has straightforward implications for prevention, diagnosis, and treatment of the disease. A genetic cause of cancer was first proposed by Boveri in 1914 (Boveri 1914). Charles & Luce-Clausen (1942) presented one of the earliest mathematical models of cancer. They studied the incidence of skin carcinomas in mice painted with a carcinogen, finding a linear relationship between the square root of the number of tumors and the time since the first painting. Their model was based on the assumption that each application of the carcinogen causes a certain number of cells to acquire mutations, but that these cells initiate abnormal growth only once both alleles of a particular gene have been mutated. The predictions of the model were in good agreement with the

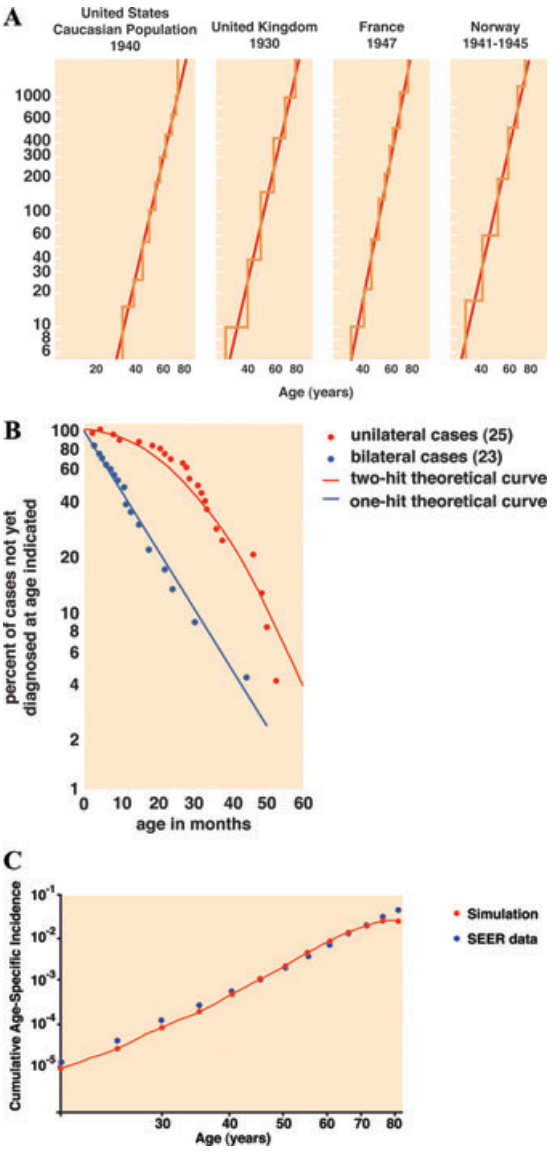


Figure 2. The study of age-specific incidence data of human cancers. (**A**) Age at death from various epithelial cancers. The plots show the number of deaths of male patients per 100,000 versus the age at death for different countries on a doubly logarithmic plane. According to Nordling (1953), the slopes of the curves indicate that the accumulation of five or six rate-limiting events is required to cause a lethal cancer; the slopes differ slightly between cancer types (data not shown). Figure adapted from Weinberg (2007). (**B**) Kinetics of sporadic and hereditary retinoblastoma. Knudson studied the retinoblastoma incidence in children and found that in hereditary cases, the percentage of cases not yet diagnosed decreases linearly with age while in sporadic cases, this percentage decreases quadratically with time (Knudson 1971). He concluded that a single somatic mutation is sufficient to cause cancer in hereditary cases and two somatic mutations are necessary for the sporadic cases. Figure adapted from Weinberg (2007). (**C**) Colon cancer incidence. The panel shows the numerical simulation of equation (1) from Liso et al. (2008) and the adjusted cumulative colon cancer incidence; data were downloaded from SEER (www.seer.cancer.gov) and adjustments were performed as in Table 1 of Michor et al. (2005a). Parameter values are mutation rate 5×10^{-4}, initial population size (i.e., number of stem cells per colonic crypt) 10, carrying capacity (i.e., final population size after clonal expansion) 10^{13}, fitness advantage of mutated cells 1%, average time between stem cell divisions 10 days, and probability of diagnosis per mutated cell 10^{-10}.

experimental data, reinforcing the notion that cancer might have a genetic cause and, in particular, could be explained by the accumulation of two mutations.

In the first half of the last century, the main source of cancer-related data was age-specific incidence. For most cancer types in adults, the number of cases per age class increases with a high power of age. Fisher & Hollomon (1951) presented a multicellular model in which mutations were assumed to occur in different cells within the same cell population and only the combination of all mutants led to cancer. Despite the dismissal of this model as being unable to convincingly explain tumorigenesis, the importance of cooperation and co-evolution of different cell types in tumors, the surrounding stroma, and the immune system has recently been highlighted (Vincent & Gatenby 2008).

As an alternative to the multicellular hypothesis, Nordling suggested in 1953 that mutations must occur sequentially in the same cell for transformation into a neoplastic phenotype (Nordling 1953). He noticed that when cancer incidence data are plotted on a doubly logarithmic plane, the resulting curve is a straight line with a slope close to six. He hypothesized that in general, cancer data with a slope of n can be explained by $n + 1$ hits (Fig. 2A). In 1957, Armitage and Doll presented a more detailed

mathematical model of the successive accumulation of mutations (Armitage & Doll 1957). They allowed for different mutation rates at different ages and assumed a fixed order in the sequence of mutations. The model was tested with age-specific mortality curves of 17 types of cancer and for patients between ages 25 and 74. For a subset of cancer types, the accumulation of seven mutations before diagnosis seemed to agree well with the observed data. Nevertheless, they noticed that a second subset did not fit the model assuming $n+1$ hits; the authors attributed this deviation to the influence of external factors such as exposure to carcinogens or the influence of endocrine secretions. These considerations led to the formulation of two distinct mathematical descriptions of cancer evolution—one assuming constant mutation rates and the other considering variable rates of mutation depending on age, sex, and the site of the disease (Armitage & Doll 1957). These authors' hypothesis that cancer results from the accumulation of multiple mutations in the same cell eventually became known as the multistage theory of carcinogenesis.

Fisher (1958) suggested that the incidence curves of most cancers can be explained by the accumulation of only three mutations. According to his approach, the slope of the incidence curve, s, is proportional to a function of the number of mutations, m, and the age t at diagnosis, $t^{3(m-1)}$. This formula was derived from a modified version of Armitage and Doll's model that included the possibility of early mutations leading to clonal expansion, thereby altering their epidemiological consequences. Fisher found that if the radius of the area covered by the mutant clone grows at a constant rate, sequential waves of faster than linear growth are expected. In the context of this model, a slope of six in the incidence curve is caused by three genetic changes, each followed by a quadratic increase in the population size of the tumor.

The next steps in the development of theories of cancer incidence were taken by Ashley (1969) and Knudson (1971). Ashley developed the first mathematical model based on a comparison of incidence data of sporadic and hereditary cancer (Ashley 1969). He studied the differences between colorectal cancer patients with and without Familial Adenomatous Polyposis (FAP), a hereditary condition now known to be caused by a germline mutation in the APC tumor-suppressor gene, which results in hundreds to thousands of polyps in the colons of teenage carriers. Ashley proposed that some of the genetic changes leading to cancer could cause increased ratios of cell division to cell death. Under this assumption, a slope of n of the incidence curve could result from either n or $n-1$ hits since the accelerated net growth rate reduces the number of necessary mutations. When comparing the incidence data of FAP with that of sporadic colorectal cancer, the difference in the slopes was found to be approximately two for both men and women. Ashley concluded that a mutation in the APC gene accounts for a slope of two or three in the incidence curve of colorectal cancer, depending on whether clonal expansion is considered. Knudson's investigation in 1971 then led to the identification of tumor-suppressor genes by theoretical techniques and became known as the "two hit" hypothesis of tumor-suppressor inactivation (Knudson 1971). The model was developed from the observation that retinoblastoma, a childhood eye cancer, presents in a hereditary unilateral version and a sporadic bilateral type. While hereditary cases accumulate linearly with age, sporadic cases increase with the second order of age in frequency (Fig. 2B). Knudson developed a statistical model predicting that two somatic mutations in an "anti-oncogene," as he called it, cause the sporadic cases, while a single somatic mutation combined with a germline mutation results in the hereditary cases. The gene later identified to cause retinoblastoma if inactivated in both alleles, RB1, became known as the first tumor-suppressor gene (Friend et al. 1986).

Inspired by Knudson's work in the 1970s, several investigations into the predisposition for and inheritance of cancer were initiated (Frank

2004a). Nunney (2003) used a probabilistic population genetics model of multistage carcinogenesis to arrive at three conclusions concerning the inheritance of cancer-related mutations: lethal or sterilizing cancers are caused by alteration of more than one gene, with the exception of retinoblastoma; cancers that occur in prereproductive ages infrequently have an inherited predisposition; and cancers depending on the mutation of several genes appear with increased incidence late in life because these cancers do not decrease reproductive fitness. Frank (2004b) developed a computational model to show that the larger the number of mutations needed to cause cancer is, the smaller the mortality rates in the population are; nevertheless, the initial mutations in the process of carcinogenesis are easily accumulated because reproductive selection cannot decrease their frequency. Frank (2005) presented a mathematical model that accounts for inherited mutations accelerating the rate of cancer initiation. He studied the age incidence of hereditary and sporadic colon cancer as well as retinoblastoma, arriving at the same conclusions as Ashley and Knudson: since cancer progression is driven by the accumulation of genetic changes, it is expected that cancers in families with inherited genetic aberrations will progress faster through the different stages of carcinogenesis than cancers in families with no inherited alterations. He emphasized that mutations that are recessive at the cellular level can nevertheless be inherited in a dominant fashion.

Fisher's work, together with papers published in recent years, demonstrated that the number of genetic changes necessary to cause cancer cannot simply be read off the age-specific incidence curve. In an effort to study the dynamics of tumor-suppressor gene inactivation, Nowak et al. (2004) designed a population genetics model and investigated how the kinetics of mutation accumulation depends on the number of cells in a compartment and the cellular mutation rates. The authors found three different laws: in small populations of cells, it takes two rate-limiting hits to inactivate both copies of the gene, while in intermediately large populations, a tumor-suppressor gene is inactivated in a single rate-limiting hit (since the rates of these hits limit the evolutionary dynamics, they are called rate-limiting). These kinetics are due to a cell with two inactivated alleles taking over the population before a cell with one inactivated allele reaches fixation; this phenomenon also emerges in different scenarios and has been called "stochastic tunneling" (Iwasa et al. 2004). In a large population of cells, it takes zero rate-limiting hits to accumulate the two mutations because the magnitude of the population size enables mutations to emerge rapidly. Therefore, a tumor-suppressor gene is inactivated with different kinetics depending on the number of cells in the population, and the epidemiological implications are not straightforward.

Further, evolutionary models investigating the incidence of leukemias have shown that a single mutation is sufficient to explain incidence curves with slopes of up to four (Michor et al. 2006a; Liso et al. 2008). A stochastic model of cancer initiation and diagnosis leads to three waiting times, the magnitude of which determines the slope of the incidence curve: (i) the waiting time until the production of the first successful mutant cell, (ii) the time for clonal expansion of its lineage, and (iii) the waiting time until diagnosis of the disease (Michor et al. 2006a). If the fitness advantage of the mutant cell is small and the population size of the tissue and/or the carrying capacity of the tumor are large, then a one-mutation model can explain incidence curves with large slopes. Hence a large slope of cancer incidence data may result from a small number of mutations together with clonal expansion of mutant lineages and the probabilistic process of diagnosis.

Despite the enormous importance of early work for establishing the multistage hypothesis of carcinogenesis, interpretations of cancer incidence curves must be done with careful consideration of the population genetics of mutations accumulating in tissues. To demonstrate the inability of cancer incidence data alone to

inform about the number of mutations necessary to cause cancer, we fit the age-specific incidence of colorectal cancer with a stochastic model assuming that a single mutation is sufficient to cause invasive cancer (model from Liso et al. 2008) (Fig. 2C). For reasonable parameter values, this model can explain the data even though it has been demonstrated that several genetic changes must be accumulated for colorectal cancer to arise (Fearon & Vogelstein 1990). Therefore, the implications of the slope of incidence data must be re-evaluated, and final proof of the number of mutations necessary to cause a particular cancer must come either from experimental evidence or from a detailed knowledge of the effects of particular mutations together with mathematical modeling. Additionally, as the investigations into the genetic causes of cancer advanced (Vogelstein & Kinzler 2002), other questions started to emerge.

The Mutator Phenotype in Cancer Initiation and Progression

In 1991, Loeb presented mathematical evidence showing that a moderately large number of mutations cannot be accumulated in the lifetime of an individual under the assumption of normal mutation rates (Loeb 1991). By multiplying the baseline mutation rate by the number of cell divisions occurring in a tissue, he showed that hundreds to thousands of cancer cells are generated within a tissue if one or two mutations are sufficient for carcinogenesis. If the accumulation of more than two mutations is necessary to cause cancer, however, then a tumor could not arise within the lifetime of an individual assuming normal mutation rates. As possible solutions to this problem, Loeb proposed mutagenic hotspots, mutational events affecting more than one gene (such as chromosome amplifications and deletions), the activity of carcinogens, mutations conferring a growth advantage to the cell, and, most importantly, a mutator phenotype. The latter hypothesis has since caused an intense debate not only in the field of cancer initiation, but also in progression and its implications in treatment and resistance. Several experimental investigations were initiated to study mutator phenotypes, leading to the characterization of two main types of genetic instabilities (Lengauer et al. 1998) (Fig. 3A): while chromosomal instability (CIN) leads to increased rates of losing (parts of) chromosomes triggered by genetic alteration of so-called CIN genes (Kolodner et al. 2002), microsatellite instability (MIN) results in elevated point-mutation rates due to a deficiency in the mismatch repair (MMR) pathway (Kinzler & Vogelstein 1996; Perucho 1996). CIN can emerge due to one dominant mutation, whereas MIN requires the accumulation of two recessive genetic changes (Fig. 3B).

Many mathematical models have been developed after Loeb's initial publication that either support or reject the hypothesis that mutator phenotypes are necessary for carcinogenesis (Tomlinson et al. 1996; Herrero-Jimenez et al. 2000; Luebeck & Moolgavkar 2002; Komarova et al. 2003; Michor et al. 2003a, 2004b; Komarova & Wodarz 2004). Tomlinson et al. (1996) published one of the first responses to Loeb's hypothesis, in which they showed that normal mutation rates can indeed produce all genetic changes necessary for carcinogenesis if fitness effects of mutations are considered. By means of computer simulations based on a simple model of cell birth and death, they demonstrated that in situations in which two mutations must be accumulated for cancer initiation, it is more likely to mutate genes that confer a fitness advantage to the cell than to additionally accumulate a mutation leading to a mutator phenotype. From this observation, the authors concluded that as long as mutations result in a growth advantage of tumor cells, genetic instability is not the main driving force of tumorigenesis—the concept of selection is sufficient to explain the onset of cancer. Nevertheless, as the number of mutations needed for initiating clonal expansion increases, the importance of genetic instability is enhanced. In particular, if six neutral mutations are needed to

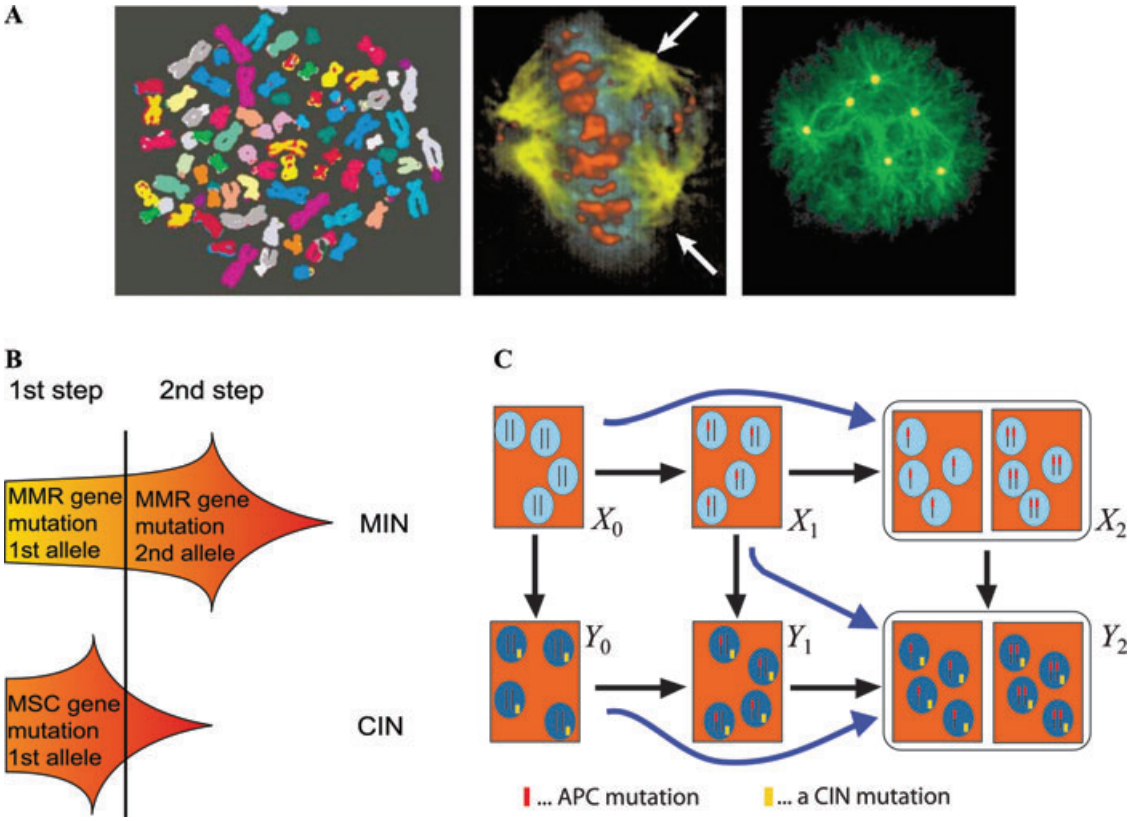


Figure 3. The mutator phenotype in cancer evolution. (**A**) Examples of mitotic abnormalities in cancer cells. Left, aneuploidy in colorectal cancer cells. Chromosomes have been painted with chromosome-specific hybridization probes, allowing the inspection of chromosome structure and function. Center, abnormal mitotic spindles in oral cancer cells. This cell was stained for kinetochore regions of chromosomes (gray), spindle microtubules (white) and chromosomal DNA, revealing an aberrant tetrapolar spindle (arrows). Right, amplification of centrosomes in prostate cancer. This cell contains six pericentrin-containing centrosomes (white dots), which nucleate multiple microtubules (gray). Figure from Jallepalli & Lengauer (2001). Online version: kinetochore regions of chromosomes (red), spindle microtubules (yellow), chromosomal DNA (green). Right, pericentrin-containing centrosomes (yellow), microtubules (green). (**B**) Pathways to genetic instability. Different types of genetic instability require different numbers of mutational hits to produce the respective instability phenotype. Top, in a heterozygote with one defective MMR (mismatch repair) allele (step 1), all that is required to begin to develop mutations at a high rate (microsatellite instability, MIN) is the inactivation of the normal allele (step 2). Bottom, chromosomal instability (CIN) has a dominant quality because a single hit (in a gene such as hBUB1, a component of the mitotic spindle checkpoint (MSC)) is sufficient to produce the CIN phenotype. (**C**) The role of CIN in tumorigenesis. The early steps of colon cancer initiation occur in small crypts, each of which is replenished by a small number of stem cells. If the effective population size of a crypt (i.e., the number of stem cells) is small, then there is a high probability that crypts contain cells of only one type at any time. Then a stochastic process can be designed that describes the evolution of the stem cell population toward a more malignant phenotype (by inactivation of the APC tumor suppressor, top row). At any time, a mutation leading to a mutator phenotype (chromosomal instability, CIN, vertical arrows) can emerge. Stochastic tunnels emerge if an intermediate cell type does not reach fixation (gray arrows). This stochastic process can be used to evaluate the chance that CIN emerges before inactivation of APC. Figure adapted from Nowak et al. (2002). Online version: Stochastic tunnels emerge if an intermediate cell type does not reach fixation (blue arrows).

initiate a neoplasm, then mutation rates must be increased by four orders of magnitude in order for a tumor to emerge within a human life span (Tomlinson et al. 1996). The effects of neutral mutations on cancer progression are further discussed below.

Herrero-Jimenez et al. (2000) presented a statistical analysis of colorectal cancer incidence data accounting for demographic stochasticity, that is, heterogeneity in the patient population due to inherited traits and environmental exposures. The authors fit a two-stage initiation–promotion model to the cancer incidence data and calculated adenoma growth rates, the number of mutations necessary for cancer initiation, and the rate of chromosome loss; the latter was found to be significantly higher than in normal cells, supporting the hypothesis of mutator phenotypes. In contrast to the findings of those authors, a similar initiation–promotion model in the hands of Luebeck and Moolgavkar (2002) led to the conclusion that genetic instability is not necessary for fitting the incidence curve of colorectal cancer. They found that two rare events (interpreted as the inactivation of both copies of APC) followed by a high-frequency event (accumulation of another mutation driving cancer progression) are sufficient to explain the incidence data. However, a scenario in which one of the rare early events is accounted for by a mutation causing genetic instability, which then accelerates the rate of APC inactivation and progression to cancer, cannot be excluded.

In 2002, a stochastic population genetics model was used to propose that the emergence of genetic instability is an early event in tumors, such as colon cancer, initiated by the inactivation of a tumor-suppressor gene (Nowak et al. 2002) (Fig. 3C). This paper led to other investigations of particular situations in tumorigenesis. Komarova et al. (2003) designed a mathematical model of the mutation-selection network consisting of the tumor suppressor APC as well as genes causing chromosomal and microsatellite instabilities, CIN and MIN. The authors found that within a broad range of parameters, a CIN or MIN mutation likely precedes the homozygous inactivation of APC. This effect is particularly pronounced if the rate of triggering genetic instability is large and the selective cost of such a phenotype is low. A subsequent paper investigated the rate of chromosome loss optimal for tumor growth that is initiated by inactivation of tumor-suppressor genes (Komarova & Wodarz 2004). Evolutionary theory predicts that higher mutation rates accelerate the rate of evolution but can lead to an error catastrophe if the rate exceeds a certain threshold (Eigen & Schuster 1977). It is therefore interesting to perform a cost–benefit analysis of large mutation rates leading to the advantages of inactivating tumor-suppressor genes as well as the disadvantages of chromosomal losses. The stochastic approach taken by Komarova and Wodarz (Komarova & Wodarz 2004) indicates that tumor initiation and progression are optimized if the rate of chromosome loss is of the order of 10^{-2} to 10^{-3} per cell division—a value that coincides with experimentally determined rates in CIN cell lines (Lengauer et al. 1997). Subsequently, Michor and colleagues presented a model in which the spatial arrangement of cells was explicitly considered (Michor et al. 2004b). Colorectal stem cells were assumed to give rise to independent lineages of differentiating cells, which, after undergoing a certain number of cell divisions, are shed into the gut lumen. By considering mutations in the APC tumor-suppressor gene and in genes causing chromosomal instability, it was found that the presence of a few genes of the latter type is sufficient to ensure that the emergence of chromosomal instability precedes the inactivation of APC. This finding showed that the hierarchical structure of colonic crypts reinforces the hypothesis of the mutator phenotype, since the organization of colorectal stem cells into small compartments reduces the protective effect of negative selection against genes causing genetic instabilities (Michor et al. 2003a).

At around the same time, several papers were published that investigated the importance and effect of mutator phenotypes in the progression

of low-grade tumors to more aggressive and invasive cancers. Solé and Deisboeck (2004) addressed the question of the existence of a mutation threshold in cancer by utilizing the quasispecies model of Eigen and Schuster (1977). Under the assumption that a mutator phenotype exists only in a subpopulation of cells, they concluded that a limited amount of genetic instability is advantageous for a cell clone; if the mutation rate surpasses a threshold, the replication rate is reduced and eventually drives the clone to extinction. The authors proposed that cancer cell populations tend to maximize both mutation and replication rates. Later on, Brumer et al. (2006) used a similar approach to study error thresholds in situations in which both CIN and MIN are present. The authors analyzed the semiconservative quasispecies model of such tumors and, considering the role of postmethylation DNA repair in tumor cells, found that CIN and MIN tumors are individually viable, while a cell containing both types of instability cannot survive. This study of error thresholds in different situations provided an explanation for experimental findings that CIN and MIN are mutually exclusive (Lengauer et al. 1998).

Michor and colleagues (2005b) extended their investigation of colorectal cancer to explore the role of genetic instability during the accumulation of all mutations considered necessary to cause invasive cancer. They found that the conditions for early instabilities are met even more easily when a larger number of mutations are considered, since the cost of accumulating a mutation causing a mutator phenotype is balanced by the larger benefit of that phenotype accelerating the inactivation of every successive tumor-suppressor gene.

One argument against the hypothesis that mutator phenotypes are necessary for tumorigenesis was that with high mutation rates, the probability of accumulating deleterious mutations increases. This effect leads to negative clonal selection of mutator phenotypes. Beckman and Loeb (2005) developed a differential equation model including considerations such as the number of cell divisions that have occurred in a tumor, the number of dominant and recessive genes conferring a fitness disadvantage, and the percentage of genomic mutations affecting them, as well as mutation rates and the number of genes present in the genome. The authors proposed that although deleterious mutations do indeed occur, the effect of negative selection is negligible since disadvantageous mutants are lost from the cancer cell population while the main clone continues to proliferate. However, other authors have suggested that most of the genes in the human genome function to constrain cellular growth and coordinate differentiation pathways (Rajagopalan et al. 2003). It might be possible, they argued, that most genetic changes arising during tumorigenesis are beneficial to a neoplastic cell. Finally, in 2007, Enderling and co-workers used a system of differential equations to emphasize the necessity of genetic instability for driving cancer progression unless the presence of a large number of stem cells and/or tumor-suppressor genes in the genome is postulated (Enderling et al. 2007).

By now it has become generally accepted that genetic instabilities play an important role in the initiation and progression of cancers. The widespread presence and clinical effects of instabilities lead to questions about pharmacological strategies that may be used to exploit this cancer trait to the advantage of patients. For example, genomically unstable tumors could be treated with agents that inflict further DNA damage on cells (such as alkylating drugs) that serve to push the tumor cells across the error threshold. However, in genetically stable tumors the amount of DNA damage incurred might be tolerable and hence could potentially accelerate cancer progression and the evolution of resistance. A more extensive theoretical investigation of optimum treatment strategies would be useful to assess the risks and benefits of DNA-damaging agents utilized to treat potentially unstable tumors. Furthermore, the number and identities of genes causing genomic instability when mutated are

still mostly unknown, and a systems biology approach to the identification of such genes, their tissue specificity, and membership of pathways is needed.

Considerations of Cancer Progression

Understanding and preventing cancer progression is one of the central goals of cancer research. The natural history of a tumor is determined not only by the genetic and epigenetic changes accumulating in the cancer cell population, but also by the tumor's interactions with the microenvironment and the immune system, as well as by the dynamics of different cell clones within the tumor.

Bodmer and Tomlinson (1995) showed that clonal expansion caused by altered cell death or differentiation rates can result in the population growing to higher plateaus in size. Using a mathematical model of discrete-time differential equations, they investigated possible scenarios for cancer growth when rates of cell death and differentiation are altered by mutations. This model was able to explain long lags in tumor progression and the existence of benign lesions growing to an equilibrium cell number. In a later contribution, d'Onofrio and Tomlinson incorporated fluctuations in the parameters and nonlinearity in the equations into the model (d'Onofrio & Tomlinson 2007). They found that fluctuations of the population size at distinct differentiation stages increase the probability of exponential growth, which is irreversible once initiated. In another extension of Bodmer and Tomlinson's work, Johnston and colleagues (2007) relaxed the need for synchronous cell divisions and presented a continuous approximation of the model. They concluded that feedback controls of the cell population add stability to the system, rendering exponential expansion impossible unless these regulatory mechanisms are altered by mutations.

The importance of apoptosis in carcinogenesis has often been emphasized (Hanahan & Weinberg 2000) and was investigated by Komarova and Wodarz with a stochastic model (Wodarz & Komarova 2007). They argued that high rates of apoptosis increase cell turnover, thus generating more mutants and increasing the probability of cancer progression. They found that an absence of apoptosis would drive the population to an evolutionary dead end, resulting in benign lesions unable to surpass fitness barriers; an optimum relationship between rates of cell death and of mutations exists such that enough mutants emerge without disruption of other pathways needed for cell viability. Also in 2007, Wodarz developed a stochastic model with which he studied the effect of cell turnover and mutational mechanisms on the processes of cancer progression and aging (Wodarz 2007). He found that if mutations occur independently of cell division, an increased cell turnover results in a higher probability of tumor progression and a lower degree of aging, therefore pointing to the existence of an equilibrium that maximizes the life span of the organism. If mutations occur only during cell division, both aging and cancer risk increase with cell turnover, thus favoring low turnover rates.

Neutral mutations have been at the center of interest of evolutionary biologists ever since Motoo Kimura introduced the theory of neutral evolution in 1968; in this landmark paper, he argued that most genetic variation is selectively neutral and hence neither subject to nor explicable by natural selection (Kimura 1968). Rather, most evolutionary change results from random drift of nonselected alleles. The emergence of neutral mutations during cancer progression and clonal evolution is of great importance since such changes could serve as evolutionary "bottlenecks" (Maley & Forrest 2001): after the initial model by Tomlinson and colleagues in 1996 (Tomlinson et al. 1996), Maley and Forrest (2001) introduced a computational model to investigate the relationship between the numbers of neutral and selected mutations as well as those changes that cause mutator phenotypes. The model was

based on computer simulations of a cell population proliferating on a two-dimensional lattice. In a large parameter search, the authors found that the number of selected mutations positively correlates with the number of neutral mutations needed to progress to cancer. Contrary to the arguments against the necessity of mutator phenotypes for cancer evolution, the authors argued that, with the normal mutation rate in human cells, a small number of selected mutations cannot be accumulated in reasonable time frames if neutral mutations are also needed for carcinogenesis.

The identification of neutral and positively selected mutants in tumorigenesis has attracted much interest since the advent of powerful genome-wide analysis tools. The observation that some genetic aberrations are present in large fractions of tumor samples of the same, and sometimes even different, cancer types suggests a defining role of the implicated genes in the process of tumorigenesis, but it has been difficult to systematically identify such "driver" mutations. To initiate a systematic analysis of genetic alterations in cancer, Vogelstein and colleagues (Sjöblom et al. 2006), as well as Stratton and colleagues (Yuen et al. 2007), determined the sequence of protein-coding genes in a total of 232 diverse human tumor samples. The studies identified 189 genes (Sjöblom et al. 2006) and 120 genes (Yuen et al. 2007) that were mutated at significant frequency. To distinguish genes likely to contribute to tumorigenesis from those in which passenger mutations occurred by chance, Sjöblom and coworkers (2006) developed statistical methods to estimate the probability that the number of mutations in a given gene is greater than expected from the background mutation rate. For each gene, this analysis incorporated the number of somatic alterations observed in a genomic mutation screen, the number of tumor samples studied, and the number of nucleotides successfully analyzed. Because the mutation frequencies vary with nucleotide type and context and are different in different tumor types, these factors were included in the calculations. The output of this analysis was a cancer mutation prevalence (CaMP) score for each gene analyzed (Sjöblom et al. 2006). The CaMP score represents the probability that the number of mutations observed in a gene reflects a mutation frequency higher than expected by chance given the background mutation rate. However, this method of estimating selection across the genome has been criticized by several authors (Forrest & Cavet 2007; Getz et al. 2007; Rubin & Green 2007; Chittenden et al. 2008). Discussions about the *P*-values used by Sjöblom and colleagues as well as their estimates of the background mutation rate have led to the suggestion that the CaMP score in its original form severely overestimated the number of driver mutations in the analyzed datasets. These criticisms were countered by the authors of the original study, who argued that the experimental setup warranted a modification of the statistical approach that, if incorporated into the other groups' models, would predict almost identical numbers of proposed driver mutations across the investigations (Parmigiani et al. 2007).

Such statistical issues cannot be adequately resolved without employing theoretical methodologies unrelated to the above techniques, as well as a functional validation of the mutations that are ranked highly by the algorithms. Such validation is essential for identifying true driver mutations and also computational techniques that correctly predict which genes are functionally relevant for tumorigenesis. In the following we review alternative approaches to the identification of functionally significant mutations in cancer.

Maley and collaborators (2004a) used a statistical approach to identify mutations conferring selective advantages in Barrett's esophagus. Biopsies sampled from different regions of patients' esophagi were analyzed for loss of heterozygosity, microsatellite shifts, point mutations, and methylation of selected loci. Genetic alterations were sorted according to the proportion of proliferating cells that carry the alteration per sample, as well as the frequency of that alteration among patients. Highly ranked

changes according to this approach were defined as driver mutations, and any genetic alteration that occurred exclusively together with such a driver was interpreted as a hitchhiker mutation. The authors found that homozygous p16 inactivation followed by p53 mutations has a strong selective advantage in Barrett's esophagus.

In 2007, Sander and colleagues presented the Online Mutation Assessor (OMA), a computational approach incorporating information about the sequence evolution and three-dimensional structures of proteins and of their interactions in macromolecular complexes, as well as their placement in molecular pathways (Reva et al. 2007). Given a nonsynonymous sequence variant, OMA provides a functional report and shows the mutated residues in the context of a protein family alignment view and a 3-D structure view. The result is a prioritized list of mutations ranked by functional score as well as background information that leads to the score, including evolutionarily conserved patterns across organisms as well as structure placement of the mutation. This algorithm is promising since it incorporates information about different aspects of the mutation and might therefore lead to a more robust identification of functionally important genes than approaches that incorporate only the frequency of mutations.

Also in 2007, Beroukhim and colleagues developed a statistical methodology called Genomic Identification of Significant Targets in Cancer (GISTIC) (Beroukhim et al. 2007). GISTIC was designed to identify significant copy-number changes in cancer genomes through two key steps: it first calculates a statistic that takes into account the frequency and amplitude of the genetic change, and then assesses the statistical significance of each genetic change by comparing the statistic derived in the first step to the results that would be expected by chance (Fig. 4A). This method identifies regions of aberration that are most likely to drive cancer pathogenesis. Since its publication, GISTIC has been applied to many genomic datasets and has led to several important findings (Weir et al. 2007; TCGA 2008). Taylor and co-workers (2008) presented a similar statistical approach, RAE, to distinguish functionally neutral from causal chromosomal alterations in tumors. The key differences between this method and earlier approaches are that RAE (i) distinguishes between four classes of genomic gains and losses to reflect the biological significances of such alterations, (ii) renders these four scoring models sample-specific, adapting to individual tumors to account for their differences, (iii) uses soft discrimination rather than hard thresholds for improved signal extraction, and (iv) generates a random aberration model using a background of segmental DNA rather than independent array markers. When the performance of RAE and GISTIC were compared (TCGA 2008), the genetic alterations that scored as statistically significant were almost identical, suggesting that both methodologies are equally good at identifying significant aberrations in cancer.

Although many different statistical and evolutionary approaches to identifying driver mutations have been proposed, the field is still lacking a comprehensive comparison of these techniques together with experimental efforts to validate purported driver mutations. Only a functional validation in cell line and mouse experiments can prove that a specific genetic alteration leads to cancer initiation or an acceleration of malignant growth. Such endeavors will be of crucial importance in the coming years.

In 2007, Beerenwinkel and colleagues developed a mathematical model to investigate the waiting time to cancer (Beerenwinkel et al. 2007). Observations of the mutational patterns in colorectal cancer led to an estimation of the number of driver mutations of around 20. Based on this hypothesis, the authors studied tumor growth and genotype dynamics and related the time for a tumor to reach a given size to the mutation rate, population size, and fitness advantage of the driver mutations. Simulations and analytical results showed that for

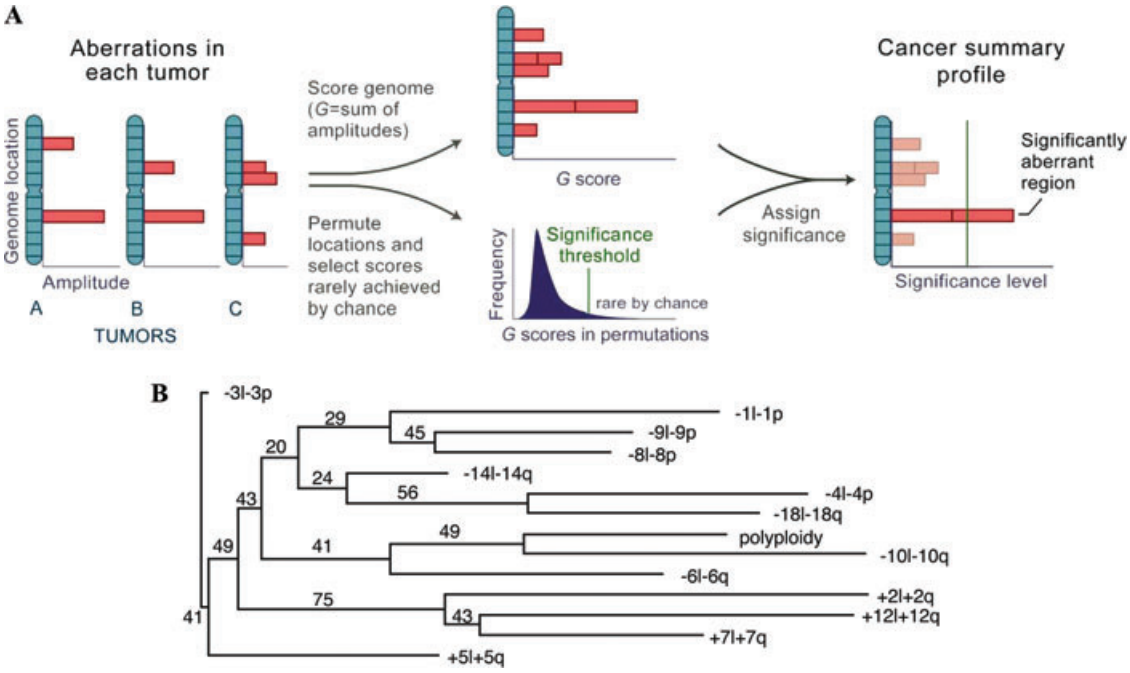


Figure 4. Identification of driver mutations and their time of emergence during tumorigenesis. (**A**) The GISTIC algorithm. After identifying the locations and magnitudes of chromosomal aberrations in multiple tumors (left), GISTIC scores each genomic marker with a score that is proportional to the total magnitude of aberrations at each location (upper center). In addition, by permuting the locations in each tumor, GISTIC determines the frequency with which a given score would be attained if the events were due to chance and therefore randomly distributed (lower center). A significance threshold (vertical line) is determined indicating the value beyond which significant scores are unlikely to occur by chance alone. Alterations are deemed significant if they occur in regions that surpass this threshold (right). From Beroukhim et al. (2007). (**B**) A maximum likelihood tree for the karyotypic evolution of clear cell renal cell carcinoma, based on the chromosomal aberrations seen in more than 10% of the cases. Deletions (amplifications) are denoted by a minus (plus) sign followed by the chromosome number and arm. Figure adapted from von Heydebreck et al. (2004).

reasonable parameter values, a fitness advantage conferred by driver mutations (assuming normal mutation rates) could account for the generation of a tumor within a human life span. The model also predicts the high level of heterogeneity seen in colorectal cancer patients, which, in the context of the model, is accounted for by the emergence of different mutations affecting the same mechanistic pathways. The authors showed that the assumption of a large number of drivers with small fitness advantages fits well with the data and the incidence of colorectal cancer. In 2008, the same group used mathematical modeling to arrive at some general conclusions about colorectal carcinogenesis (Jones et al. 2008): (i) it takes about 17 years for a large benign lesion to evolve into cancer but less than 2 years for cells within that cancer to acquire the ability to metastasize; (ii) it takes few, if any, selective events to transform a highly invasive cancer cell into one that has the ability to metastasize; and (iii) the rates at which point mutations develop in advanced cancers are similar to those of normal cells. Such hypotheses, even though interesting, require experimental validation and remain a theory without wet-lab efforts.

It is of interest not only to identify all driver mutations implicated in a particular cancer, but also to uncover the temporal sequence in which these mutations arise. The earlier a driver mutation emerges during tumorigenesis, the more

likely it is to induce "oncogenic addiction" (the dependence of a cancer cell on the activity of a particular oncogene) and therefore could represent the most promising drug target (Weinstein 2002). In 1990, Fearon and Vogelstein published their paradigmatic result regarding mutational pathways, suggesting that the accumulation of a linear series of genetic changes transforms colonic tissue into invasive cancer (Fearon & Vogelstein 1990); however, this so-called "Vogelgram" has since been criticized for describing the tumorigenetic processes of only a subset of colorectal cancers (Smith et al. 2002). The identification of evolutionary trajectories toward cancer has become a hot topic in theoretical research, particularly because the experimental conditions under which Fearon and Vogelstein's results were obtained are hard or even impossible to reproduce for other cancer types.

A few years later, Desper and colleagues (1999) presented one of the first theoretical approaches to this question. They introduced branching mutational trees—so-called oncogenetic trees—to the investigation of cancer progression. The method was applied to cross-sectional comparative genomic hybridization (CGH) data obtained from tumor samples. The goal of the algorithm was to identify the tree that best describes the observed joint probabilities of each pair of mutations. Their use of tree reconstruction methods allows for the formulation of temporal relations between genetic changes and can lead to the identification of the order in which mutations occur. One drawback to the approach of Desper and colleagues is that events in the leaves of the tree can only occur once all previous events have happened, resulting in large portions of the tree with zero likelihood of occurring. To overcome this problem, Beerenwinkel and coworkers (2005) extended Desper's algorithm by introducing mixture trees. These trees combine suboptimal trees, including a special topology that contains all events independently; due to this modification, all genetic alterations can occur without depending on previous mutations. It was shown that mixture trees could be used to investigate independent mutational pathways, which were enigmatic when using single-tree reconstruction. The authors also developed a measure of progression to estimate the survival times of individual patients and validated their predictions with clinical datasets. In 2002, Newton developed a method to find combinations of genomic aberrations from CGH data (Newton 2002). This approach involved a joint probability distribution of the samples' profiles from which ensembles of genomic abnormalities were inferred. One important characteristic of the model is that no preselection of relevant genetic abnormalities is necessary, since such changes are automatically inferred by the algorithm.

A variation of these tree reconstruction methods was presented by von Heydebreck and colleagues in 2004 (von Heydebreck et al. 2004). In their model, the leaves of the tree represent mutational events while intermediate nodes denote "hidden" events, which might be interpreted biologically as intermediate (genetic) events (Fig. 4B). Starting with the wild-type node, stochastic experiments are realized that evolve according to the joint probabilities between events. The highest-scoring tree is constructed using a likelihood-maximization algorithm, resulting in a tree topology with branch lengths representing the probability to move from one node to the other; the tree therefore contains information about the likely temporal occurrence of events in a mutational pathway.

Even though these phylogenetic algorithms promise to reconstruct the temporal sequence in which driver mutations arise during tumorigenesis, they have not been validated using the one dataset for which the answer is known: colorectal tumorigenesis. Once an algorithm has been validated with available data, the predictions of the model should be tested in *in vivo* models to investigate the stage at which the mutation arises experimentally as well as the effects it has on cell turnover, physiological dependencies, and other issues.

Finally, in 2006, Michor and colleagues developed stochastic mathematical models to investigate the dynamics of metastasis formation in patients (Michor et al. 2006c; Michor & Iwasa 2006). The authors considered situations in which one or two mutations–either single activating mutations in genes such as rat sarcoma virus homolog (RAS) and myelocytomatosis viral oncogene homolog (MYC) (Michor et al. 2006c) or inactivation of both alleles of metastasis-suppressor genes like Metastasis inhibition factor (NM23) (Michor & Iwasa 2006)—are necessary to enable cells to metastasize. They considered the dynamics of metastasis-enabling mutations in a cell population of constant size and investigated whether metastatic potential is the property of all cells in the primary tumor or of only a small subset. They found that if most metastases are caused by mutations that confer a fitness advantage to the cell in the primary tumor, then most or all cells in the primary tumor will carry metastasis-enabling mutations; this effect arises because, in that case, a mutant cell is likely to reach large frequencies in the tumor. In contrast, if most metastases derive from disadvantageous mutations, then only a small fraction of cells in the primary tumor will have metastatic potential. To compensate for the selective disadvantage in the primary tumor, such mutations must be able to successfully found metastases elsewhere with a probability that is many orders of magnitude higher than that for advantageous mutations. Since this scenario is unlikely, the authors conclude, most metastases should arise from mutations that have reached a large frequency in the primary tumor. This finding is supported by experimental evidence (Ramaswamy et al. 2003). These models were later extended to situations in which the growth of the main tumor is described by a branching process and the tumor may undergo exponential expansion (Dingli et al. 2007a).

Evolutionary approaches to invasion and metastasis are still underrepresented in the literature, and many open questions remain. Is metastatic ability conferred exclusively by genetic changes, or do other cellular adaptations provide for such capabilities? Can a mathematical model incorporating mechanical forces within a tumor explain the patterns of metastasis seen in patients? How can the evolution of metastatic sites best be prevented by therapies? Future theoretical investigations aimed at reducing the mortality associated with invasion will be useful for the clinical management of late-stage cancer.

Stem Cells, Differentiation, and Heterogeneity

The role of tissue-specific stem cells, differentiation hierarchies, and the resulting tumor heterogeneity in carcinogenesis has attracted the interest of both experimentalists and theoreticians (Fig. 5). Tissues are organized into differentiation hierarchies of cells: the most primitive cells are pluripotent stem cells capable of proliferation, self-renewal, and the production of many types of differentiated progeny (Reya et al. 2001). Stem cells produce committed progenitors, which in turn produce even more committed cells. Differentiated cells typically proliferate to fulfill organ-specific tasks. Once fully differentiated, however, such cells may lose the ability to replicate, as illustrated by the loss of nuclei in erythrocytes and keratinocytes (Bach et al. 2000). In fact, the organization of tissues into hierarchical structures might have evolved to minimize the risk of carcinogenesis, since stem cells are more prone to accumulating mutations that lead to cancer than differentiated cells due to their enhanced self-renewal capabilities and should therefore represent only a small fraction of cells in a tissue (Michor et al. 2003b).

Cairns (1975) was the first to put forward the hypothesis that stem cells retain an "immortal strand" of genetic material. He proposed that to keep from accumulating mutations, stem cells could divide asymmetrically, retaining the original strand of DNA while passing the newly synthesized, and possibly mutated, strand to a

daughter cell. This hypothesis has since led to controversy, with Conboy and associates providing supporting evidence by showing template strand cosegregation in muscle stem cells (Conboy et al. 2007), and Kiel and colleagues (2007) insisting that hematopoietic stem cells do not asymmetrically segregate chromosomes. This controversy awaits resolution.

In 2002, Cairns presented a mathematical model of cancer initiation based on the assumption that mutant stem cells undergo apoptosis due to deficient DNA repair mechanisms and are replaced by progenitors that may have accumulated mutations (Cairns 2002). His set of simple formulas showed good concordance with observed tumor incidence of mice treated with carcinogens. Cairns argued that the biological features represented in his model could explain the onset of cancer even with the low mutation rates of human cells.

Recently, Pepper and colleagues presented a model of cell differentiation and somatic evolution. By means of a system of differential equations, the authors studied the effects of mutations as well as changing replication and differentiation rates in a hierarchically structured population (Pepper et al. 2007). Several conclusions were drawn: carcinogenesis can be initiated both in stem cells and transient-amplifying cells, genetic lesions disrupting differentiation pathways are critical to tumorigenesis and should be considered among the most fundamental hallmarks of cancer, and the structure of serial differentiation is a general strategy for the suppression of somatic evolution in tissues.

Adding to the importance of differentiation structures is the fact that long lineages increase the probability of accumulating mutations, since there are more possibilities for mutations to arise if the number of cell divisions a clone has undergone is large. Frank and colleagues (2003) developed a model to quantify the risk of cancer initiation for different tissue architectures. The authors found that, if the mutation rates of stem and differentiated cells are of comparable magnitude, a tissue structure with a shorter lineage has a reduced risk of cancer. If the mutation rates of stem cells are significantly smaller than those of progenitors or differentiated cells, then the risk of cancer depends on the number of mutations necessary to drive tumorigenesis: for large numbers of mutations, more transit and fewer stem cell divisions are favored. As the number of mutations decreases, a structure with shorter lineage has a decreased risk of cancer. In the same year, Nowak and co-workers presented a linear model of mutation accumulation that takes the spatial relationship between cells explicitly into account (Nowak et al. 2003). Using stochastic processes, the authors showed that in such a spatial model, fitness differences between non–stem cells are unimportant since they are "washed out" of the system, leaving stem cells as the only source of lasting variation (Fig. 5B). The authors also concluded that such spatial organization results in an overall reduction in the probability of cancer initiation. Later on, Dingli and colleagues investigated the effects of stochasticity on the dynamics of stem cells given the small number of replicating cells in a compartment (Dingli et al. 2007b). They showed that remission of tumors and rapid expansion of mutant clones could both be explained by means of stochastic dynamics alone.

Such theoretical investigations of differentiation structures provide important insights into the evolutionary effects of tissue design. However, in the absence of a clear connection to experimental results, these endeavors remain abstract. It would be interesting to see mathematical approaches to situations in which differentiation is disrupted by specific mutations. Such investigations can lead to suggestions of therapeutic intervention based on predicted effects of perturbations of the system.

Genetic and phenotypic heterogeneity of tumors is not only caused by differentiation hierarchies, but also by cancer progression (Maley et al. 2006), mutator phenotypes and the evolution of resistance (Komarova & Wodarz 2003), and interactions of cancer cells with their microenvironment (Gatenby & Vincent

2003a) (Fig. 5A). Historically, special emphasis has been devoted to studying the mechanisms of generation and maintenance of such heterogeneity, and a number of computational approaches have been developed. In 2002, González-García and colleagues investigated a stochastic spatial model of tumor growth and showed that coexistence of diverse clones with different fitness values in the same tumor is possible due to the complexity of the microenvironment (González-García et al. 2002). The evolution of a tumor in three-dimensional space was simulated utilizing simple mutation–selection rules. The authors showed that maintenance of diversity can be explained by spatial dynamics since the exclusion of competitors takes a long time as compared to the time scale of tumor growth. A similar approach was used by Zhang and colleagues (2009). They implemented a three-dimensional model of tumor growth as a computer simulation and considered several levels of environmental interactions. They investigated the accumulation of a linear sequence of mutations, each capable of changing the phenotypic response of the cell to the environment. The model predicted considerable heterogeneity at the molecular level and different rates of evolution in distinct regions of the tumor. For instance, in those clones located near blood vessels, diversity was reduced due to the expansion of fast-growing phenotypes which led to an increased rate of progression to more aggressive variants; regions close to the core of the tumor show a sustained increase in diversity since in that environment coexistence of different clones is possible. In 2004, Nagy designed a differential equation model to describe three aspects of a solid tumor: change in cell mass over time, change in tumor vascularization over time, and competition between two different cancer cell types (Nagy 2004). The model also describes the number of immature vascular endothelial cells, which build blood vessels. The model predicts that natural selection always favors more aggressive cancer cell phenotypes, even if the growth of such phenotypes eventually destroys all or part of the tumor. The author suggested that this "hypertumor" mechanism may be the cause of necrosis observed in many vascularized tumors. A similar mechanism was later applied to study tumor incidence across species (Nagy et al. 2007).

In 2006, Maley and colleagues demonstrated for the first time a direct relationship between clonal diversity in premalignant lesions and progression to cancer (Maley et al. 2006). They investigated the heterogeneity of cellular clones in patients with Barrett's esophagus using ecological measures of diversity, finding that diversity was highly correlated with risk of progression to esophageal carcinoma (Fig. 5C). Diversity was measured by three different indices, which incorporated information about loss of heterozygosity (LOH), microsatellite shifts, and sequence mutations across the genome. A high level of clonal diversity was found to increase the risk of progression to malignancy, but to stem from genetic instability only if such variation leads to viable phenotypes. Normal apoptotic pathways may thus prevent progression of the tumor by limiting population diversity and eliminating cells with a mutator phenotype.

Theoretical investigations of this nature are important both for an evolutionary understanding of tumorigenesis as well as for a guideline for clinical interventions: it is important to predict which patients with premalignant lesions are most likely to develop malignant tumors such that the risks of cancer and of unnecessary surgery can be minimized. Unfortunately, few systems are amenable to approaches relying on measurements of premalignant tissue. However, it is an urgent goal of the field to identify methodologies and histologies in which such important studies can be performed.

In 2008, Vincent, Gatenby, and Gillies proposed that cancer cells must surpass several environmental barriers in order to acquire an aggressive–invasive phenotype (Vincent & Gatenby 2008; Gatenby & Gillies 2008). The authors presented an evolutionary game theory model to investigate the characteristics of

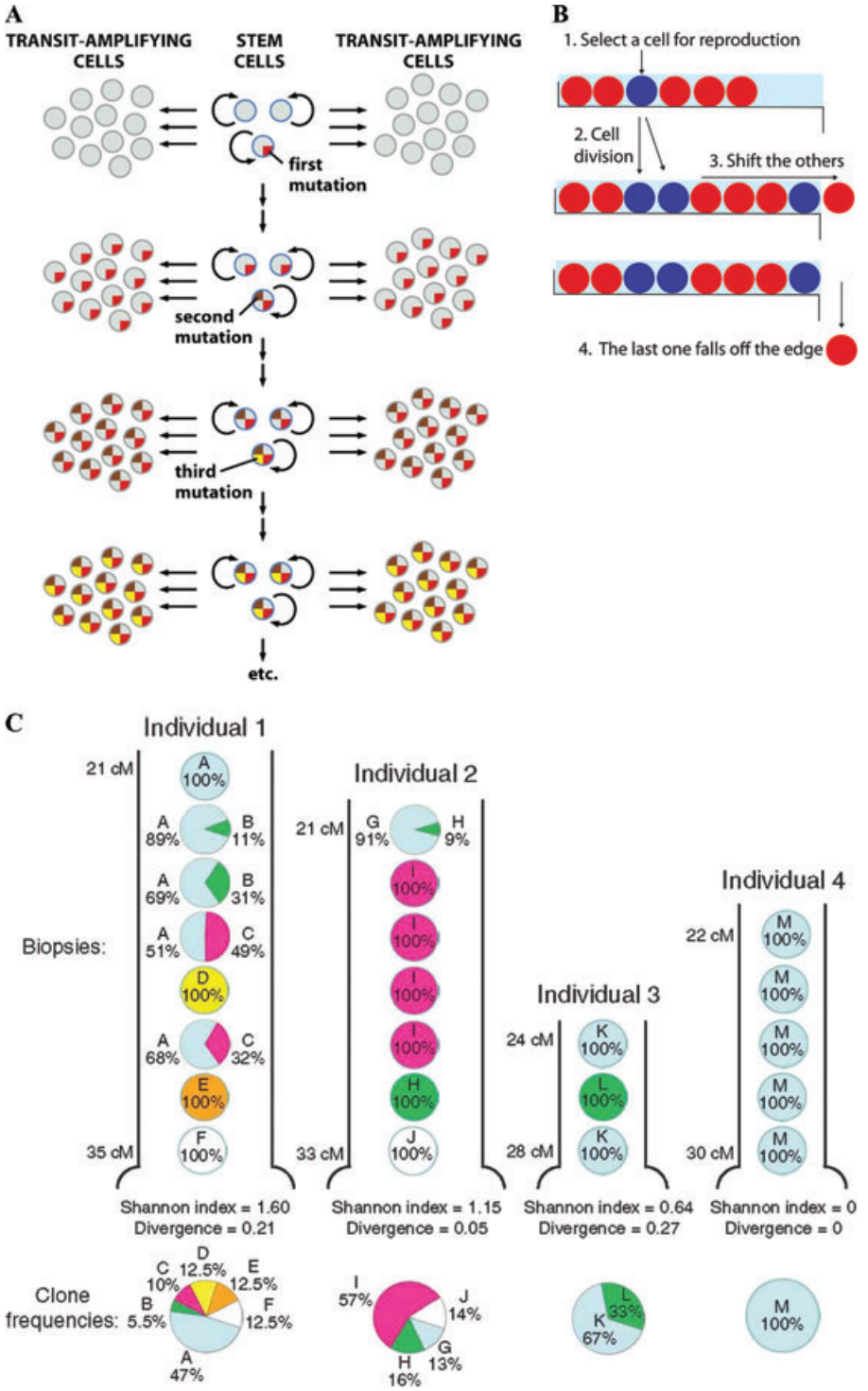


Figure 5. Differentiation hierarchies, spatial arrangements, and heterogeneity in cancer. (**A**) Cancer stem cells and clonal succession. Cancer stem cells (middle) can differentiate into transit-amplifying cells (horizontal arrows), but can also accumulate further mutations that drive cancer progression (vertical arrows). Both differentiation and progression processes contribute to tumor heterogeneity. From Weinberg (2007). (**B**) "Linear process" of somatic evolution.

tumor initiation, promotion, and progression (Vincent & Gatenby 2008). Their analysis was based on the idea of adaptive landscapes, in which selective traits are altered as the microenvironment changes; the microenvironment is in turn shaped by the evolving populations of cells. Selective barriers arise from cell–cell interactions, tissue organization, blood supply, the stage of carcinogenesis, and other intra- and extra-cellular factors. Numerical simulations coincided with the observed biological features of a multistep process: initially, mutations occur mostly in tumor suppressor genes—these mutations are selectively (almost) neutral but set the fitness landscape for the second phase of carcinogenesis, in which limited proliferation is initiated. This slow expansion is surpassed only in the third stage of carcinogenesis when glycolytic and acid-resistant phenotypes evolve. The authors concluded that normal tissue is situated in an adaptive landscape, which allows for the coexistence of multiple cell types—a condition needed for the evolution of multicellularity. While normal conditions in this landscape impose strict limitations on proliferation, populations of benign cells are vulnerable to invasion of fitter genotypes, which initiate tumor growth. Further progression is limited by available blood supply, but accelerated by promoters such as inflammation or wounding which can increase the flow of nutrients, as well as mutations leading to adaptations to acidic–hypoxic environments. The authors proposed that the stages of tumorigenesis strongly depend on changes in tumor microenvironment, rather than exclusively on variation of intracellular processes such as cell cycle regulation or apoptotic mechanisms. In a similar setting, Gatenby and Gillies made several important observations: the environment selects for phenotypes instead of genotypes, the full cancer phenotype is attained only once all the barriers have been surpassed, and adaptation to one barrier may change the adaptive fitness response to subsequent ones (Gatenby & Gillies 2008).

Basanta (2008) devised a model based on evolutionary game theory to analyze the interactions between three different tumor cell phenotypes defined by autonomous growth, anaerobic glycolysis, and tissue invasion. He found that the invasive phenotype is more likely to evolve after appearance of the glycolytic phenotype, which would explain the presence of invasive growth in many malignant tumors. These results suggest that therapies which increase the fitness cost of switching to aerobic glycolysis might decrease the probability of emergence of more invasive phenotypes; when applied to glioma progression, the model explains aspects such as the emergence of diffuse tumor cell invasion in low-grade tumors.

The application of concepts from evolutionary game theory to cancer enables researchers to investigate the effects of frequency-dependent fitness on tumorigenesis. Such models may be superior to approaches using frequency-independent fitness in situations

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In this process, cells are arranged in a row and labeled $i = 1, \ldots, N$. For reproduction, cells are chosen proportional to their reproductive rate. The reproducing cell is replaced by two daughter cells, and all cells to the right are shifted by one position. The right-most cell undergoes apoptosis (falls off the edge of the one-dimensional table). This architecture can delay the onset of cancer since only mutations arising in the left-most cell, the stem cell, remains in the population. From Nowak et al. (2003). (**C**) Clonal diversity in individuals with Barrett's esophagus. Barrett's esophagus is an abnormal change in the cells of the lower end of the esophagus thought to be caused by damage from chronic acid exposure and is considered to be a premalignant condition associated with an increased risk of esophageal cancer. It is important to identify those lesions likely to progress to cancer since unnecessary surgery should be avoided. In the panel, we show genetically distinct fractions of a biopsy with their frequencies. Clones and total frequencies within a segment were used to calculate the Shannon diversity index and mean pairwise genetic divergence scores. Such measures of diversity are able to predict the risk of progression to esophageal cancer. From Maley et al. (2006).

in which the cellular growth rate or some other cell-specific parameter is a function of the behavior of other cells. For example, scenarios in which some cells secrete substances that are beneficial to the population as a whole but costly to produce for the cells themselves (such as production of vascular endothelial growth factor [VEGF] to initiate vascularization) require game-theoretic approaches (Axelrod et al. 2006). However, for some situations arising in cancer it has not been found that a trait depends on the composition of the population. In those cases, the consideration of frequency-dependent fitness does not lead to appreciable additional insights and should be neglected due to unnecessary complexity.

The investigation of cellular heterogeneity in tumors has led to many insightful results regarding its emergence, maintenance, and pathological importance. The interest of investigators in tumor heterogeneity was further piqued by the clinical phenomenon of drug resistance, and many models were developed to study the dynamics of tumors during treatment and the kinetics of resistance mutations.

Anti-Cancer Therapy and the Evolution of Resistance

The first wave of contributions to mathematical modeling of cancer treatment was started in 1964 by Skipper and colleagues who investigated the response of murine leukemias to chemotherapy, establishing the so-called "log kill" law: they showed that in an exponentially growing population, the fraction of cells killed by a drug is independent of the total number of tumor cells (Skipper et al. 1964). Thereafter, other models of anti-cancer therapy were developed to estimate tumor growth rates and to optimize chemotherapeutic dosing schedules (Norton & Simon 1977). The investigation of cancer therapy from an evolutionary viewpoint, however, was initiated only after Goldie and Coldman started thinking about the emergence of resistance in the 1980s (Goldie & Coldman 1983, 1984; Coldman & Goldie 1986). In 1983, they presented a mathematical model of cancer treatment to investigate the risk of resistance, and found that the probability P of having a resistant cell at any given time is a sigmoid function of the number of cells in the tumor, N, and the mutation rate, α, given by $P = 1 - \exp[-\alpha N]$ (Goldie & Coldman 1983). The shape of this function plotted against the logarithm of the population size is constant across mutation rates—once the probability of cure starts decreasing from the initial plateau, small delays in the start of treatment lead to significant reductions in the chance of successful treatment (Fig. 6A). The authors proposed two strategies to maximize the probability of successful therapy: first, treatment must be started as soon as possible. This conclusion was drawn from the fact that the probability of cure decreases as the size of the tumor increases, and also from the prediction that larger tumors present a higher level of heterogeneity, which is inversely related to the probability of successful therapy (Goldie & Coldman 1984); second, multiple drugs should be used in combination whenever possible, or otherwise alternated. Assuming that the growth rate of sensitive cells is decreased by the effect of the drug, short treatment breaks to limit the toxicity ensure that clones do not reach large abundances when the drug is not administered. The authors also utilized stochastic models of tumor growth to calculate the risk that clones with multiple mutations emerge; their approach lead to the suggestion of strategies maximizing the probability of successful therapy (Coldman & Goldie 1986). The prediction that early initiation of therapy and the use of multiple drugs could reduce the risk of resistance became known as the Goldie-Coldman hypothesis.

Coldman and Murray presented in 2000 a stochastic model to investigate the optimum administration strategies of chemotherapeutic drugs for different tumor growth dynamics (Coldman & Murray 2000). They considered situations in which a patient is treated with two

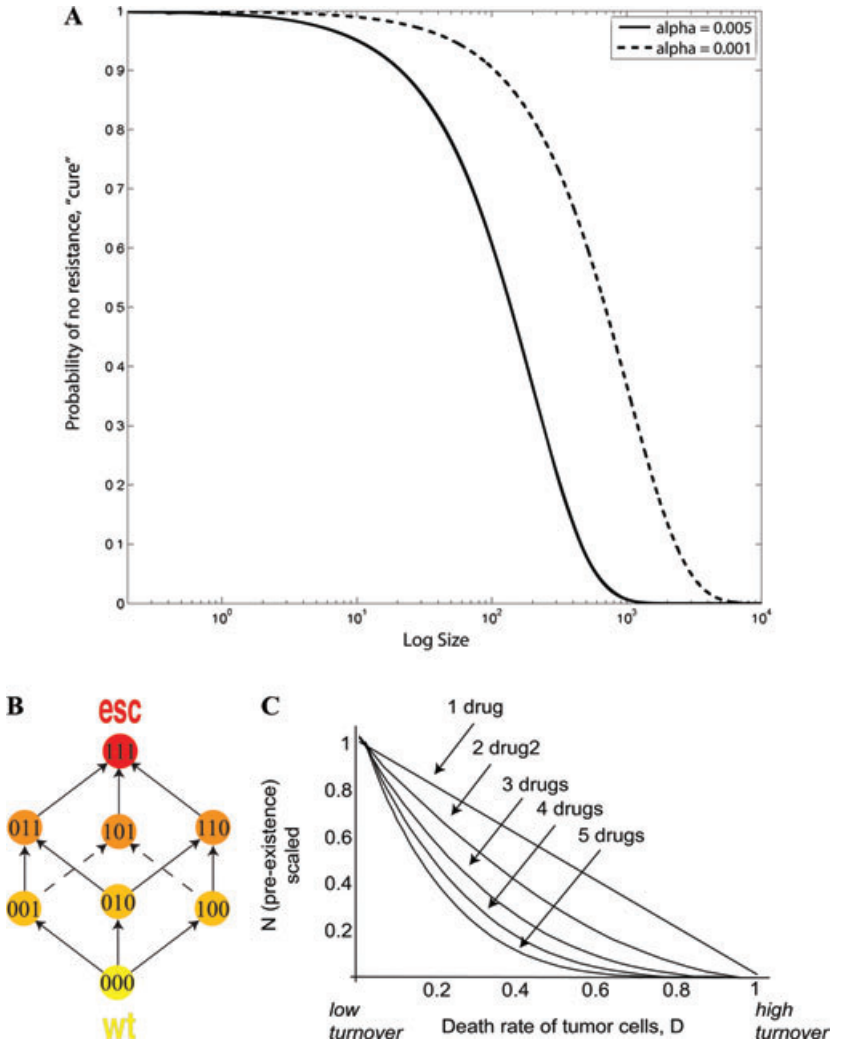


Figure 6. The evolution of resistance to anti-cancer therapy. (**A**) The mathematical investigation of resistance mutations was initiated by Goldie and Coldman, who found that the probability of cure depends on the mutation rate and the population size of the tumor and is given by the exponential of the negative mutation rate times the population size. The panel shows the probability of cure for two different mutation rates, A and B, versus the logarithm of the population size. Equation from Goldie & Coldman (1983). (**B**) A stochastic process model can be used to investigate the risk of resistance emerging in a mutation-selection network. If, for example, three positions must be mutated for resistance to anti-cancer therapy and the three mutations can be accumulated in any order, then the evolutionary trajectories from a wild-type cell (three unmutated positions, 000) to an escape mutant (three mutated positions, 111) can be studied and the risk of resistance quantified. From Iwasa et al. (2003). (**C**) The probability of producing resistant mutants before treatment depends on the death rate of tumor cells, *D*. In this example, the mutation rate is set to zero once treatment is started, and mutants are assumed not to die. The tumor size, *N*, is shown at which the probability of treatment failure due to preexisting resistance equals 0.01. While the dependence is linear for a single drug, it becomes stronger with an increasing number of drugs. Here the mutation rate is 10^{-6} and the growth rate of cells is one. From Komarova & Wodarz (2005).

drugs and assumed that mutations conferring resistance to one drug are sensitive to the other. The authors optimized drug dosage strategies to reduce toxicity and increase the probability of cure, showing that for equally effective drugs, the optimum treatment regimen consists of combined symmetric doses administered as often as possible. The model predicts that an early control of resistant clones—attained by high initial doses followed by a decreasing dose for the remainder of the treatment time—is important. Iwasa and colleagues (2003) designed a stochastic mathematical model based on multi-type branching processes to investigate the evolutionary dynamics of resistance against anti-cancer therapy. They considered a scenario in which a heterogeneous population of cancer cells is subjected to chemotherapy, and calculated the probability of success or failure of biomedical interventions consisting of one or more drugs (Fig. 6B). They found that the chance of cure is largest when many drugs are administered simultaneously since only if tumor cells evolve many mutations will they be able to escape from eradication by therapy.

The scenarios investigated in these contributions represent just a subset of the situations that can emerge when tumor cells evolve drug resistance. Other scenarios include the treatment of a cancer patient with a cocktail of drugs that contains both specific (molecularly targeted) and unspecific (general cytotoxic) agents, the necessity of altering several molecular pathways for a cell to evade chemotherapy and immune attacks, dose-limiting toxicity and side effects, and the emergence of radiation- or chemotherapy-induced secondary tumors. The literature on models optimizing therapeutic strategies for such more complicated scenarios is still small, and it is essential to obtain a quantitative understanding of anti-cancer treatment strategies and their long-term risks and benefits such that drug resistance can be prevented; the field is in dire need of optimization studies guiding therapy decisions.

Komarova and Wodarz (2003) investigated the effects of mutator phenotypes on the risk of resistance by considering parameters like apoptotic efficiency, the cost of DNA repair, and the magnitude of the mutation rate. Their stochastic model predicted that genetic instability is positively selected for in clones with low mutation rates since that phenotype confers enhanced evolvability to cells; in situations in which mutation rates are high, however, genetically stable cells experience a selective advantage since cells with a mutator phenotype accumulate deleterious mutations at a high rate. The authors found that the outcome of the system depends on two parameters: the difference in the ability to repair DNA damage between stable and unstable cells and the cost of generating lethal mutations due to genetic instabilities. In the same year, Gatenby and Vincent combined population biology and evolutionary game theory to study the dynamics of tumor and healthy cells when treated with cytotoxic drugs (Gatenby & Vincent 2003b). A model reminiscent of Lotka–Volterra equations was used to describe the interactions of normal and cancer cells interpreted as a predator–prey system, and the authors found that, in general, treatment with cytotoxic drugs alone is insufficient to eradicate the tumor. This model was used to identify two main barriers to achieving a complete remission of the tumor: evolving populations tend to produce resistant clones, which are able to drive the system back to equilibrium abundance, and drug-induced alterations of the tumor microenvironment change the selection pressure and select for tumor cells with larger evolvability.

The existence of resistance mutations at the time of diagnosis has been of considerable interest to the cancer research community since such pre-existing resistance influences treatment choices. The investigation of resistance mutations emerging while a population undergoes exponential expansion was initiated by Luria and Delbrück in 1943 (Luria & Delbrück 1943). They were interested in the mutations of bacteria that confer resistance to phages and performed experiments as well as calculations to quantify the rate at which such

mutations emerge during bacterial growth. Their analytical results describing the distribution of the number of mutants in an exponentially growing population became known as the Luria–Delbrück distribution. This distribution is also useful for situations arising in cancer and has been studied for more than half a century (Skipper 1983; Sarkar 1991; Zheng 1999; Angerer 2001; Frank 2003; Dewanji et al. 2005; Iwasa et al. 2006; Haeno et al. 2007; Komarova et al. 2007; Zheng 2008). Several different models have been suggested that are based on pure birth processes and do not include the possibility of cell death (Zheng 1999). However, in most examples of cancer growth, cancer cell death cannot be neglected.

In 2003, Frank developed a branching process model to investigate the distribution of cells with different numbers of mutations (Frank 2003). He found that the distribution of cells with two mutations provides information about the effective time of occurrence of the first mutation; the latter—depending on its frequency in different tissues in the body—could even have arisen during embryonal development. The time at which the first mutation emerges contains information about the number and types of tissues harboring this mutation, which in turn influences cancer prognoses and provides information about tissue specificity effects. In 2005, Dewanji and colleagues developed an extension of the Luria–Delbrück model that considered non-exponential growth dynamics (Dewanji et al. 2005). The authors found that including the possibility of cell death, as well as the assumption of Gompertzian growth of mutant cells, leads to larger variations in the number of cells harboring resistance mutations.

Iwasa and co-workers (2006) designed a branching process model to calculate the probability of resistance mutations existing at the time of diagnosis, as well as the expected number of resistant cells, as a function of detection size and mutation rate. These authors also explicitly considered cell death. They concluded that the probability of resistance is an increasing function of the detection size times the mutation rate, and that a tumor with larger apoptosis rates has a higher incidence of resistance. Haeno and collaborators (2007) extended this stochastic model to incorporate resistance due to the accumulation of two mutations and arrived at similar conclusions. Later Komarova and colleagues (2007) presented a variation of the Luria–Delbrück model for a stochastically growing population of cancer cells. As in prior papers, it was shown that a higher cell-death rate leads to a larger number of mutants. Furthermore, irreversible mutations were found to act as a selective force in large cell populations even if they do not confer a fitness advantage to the cell; this effect results from considering unidirectional mutations, which continuously reduce the pool of unmutated cells.

These investigations of resistance mutations existing prior to diagnosis are important for a quantification of the risk of resistance as well as for deciding on appropriate treatment choices at diagnosis. Unfortunately, cell-specific parameters such as growth and death rates and mutation rates are unknown for most cancer types. To adequately predict the risk of pre-existing resistance for a given tumor type, quantitative measurements of these parameters are essential. It is therefore import to perform *in vitro* experiments to determine these rates.

In 2005, Komarova and Wodarz developed a stochastic mathematical model to investigate treatment strategies involving multiple drugs (Komarova & Wodarz 2005). The effect of therapy was modeled by increasing the ratio of cell death and cell birth in cancer cells. The authors assumed that a single mutation is sufficient to confer resistance to one particular drug; hence, resistance against therapies consisting of n different drugs requires the accumulation of n mutations. The authors investigated the stochastic process model for situations with different magnitudes of cell turnover and evaluated the importance of resistance mutations emerging during treatment with multiple drugs. They found that the treatment phase is unimportant for the production of resistant cells and can

be neglected when determining the total risk of resistance—the majority of resistance mutations arise before the tumor is diagnosed. The chance of resistance increases with enhanced turnover rates independently of the number of drugs administered. The model was also used to find the optimum number of drugs such that both toxicity and the evolution of resistance are minimized; this number depends on the growth and death rates of cells, the mutation rate, and the population size of cancer cells at the start of therapy (Fig. 6C). When the model was applied to chronic myeloid leukemia (CML) to investigate the optimum number of drugs, it was found that administration of three chemotherapeutics minimizes the risk of resistance. Later on, Komarova and Wodarz studied the importance of stem cell quiescence in the evolution of resistance against anti-cancer treatment (Komarova & Wodarz 2007). The authors used a stochastic process model to investigate the dynamics of a population of cancer stem cells that cycle between an active, proliferative state and an inactive, quiescent state. The model was applied to CML and could reproduce the biphasic decline in blood counts seen in CML patients (Michor et al. 2005a), which in the context of this model was explained by the differential response of cycling and dormant stem cells to treatment with the targeted agent Gleevec. The authors also predicted that the probability of resistance would be independent of the presence and extent of quiescence if therapy involves only a single drug. When two or more drugs are used, the risk of resistance increases with the percentage of quiescent stem cells. In line with previous results obtained by these authors, the risk of resistance emerging during therapy is negligible when compared with resistance arising before the start of treatment. These findings have important implications for the design of treatment strategies since that showed that the use of drugs that reduce the number of quiescent cancer stem cells cannot decrease the chance of resistance effectively.

The effect of quiescence on the risk of resistance is an important phenomenon to consider, and there are other situations in cancer that should also be addressed. For instance, it is of interest to study the evolutionary dynamics of cancer cell resistance when migration and adaptation to foreign environments are included; a change in microenvironment might lead to modifications in gene expression, which can in turn modulate the risk of resistance. Furthermore, the interaction between tumor cells, stroma, immune system cells, and vascular endothelial cells may alter the risk of resistance. Future studies of resistance dynamics should address these more complicated situations.

While most mathematical models have focused on the action of cytotoxic or targeted drugs whose objective is to kill cancer cells, Maley and colleagues recently studied the possibility of using benign cell "boosters" to increase the fitness of healthy cells (Maley et al. 2004b). The authors developed a computational model to investigate the dynamics of mutations emerging in cells that proliferate on a two-dimensional lattice. In this model, the genetic information of cells is represented by a small number of loci acting as oncogenes, tumor suppressor genes, genes preventing mutator phenotypes, and genes conferring sensitivity to drugs. The population size is assumed to be constant, thus exploiting competition among different clones. The authors showed that therapies that increase the fitness of benign cells, so-called boosters, are effective in all stages of the disease when combined with traditional chemotherapy. They also suggested that increasing the fitness of chemosensitive cells prior to treatment would improve treatment outcomes. In a different approach to cancer therapy, Wodarz designed a mathematical model to investigate the use of viruses as anti-tumor agents (Wodarz 2001). He studied a system of differential equations for three possible scenarios of the interaction between virus particles and tumor cells, arriving at predictions about the necessary conditions for successful therapy. Such alternative approaches to treating cancer are worth

exploring and should be investigated with quantitative techniques.

Conclusions and Outlook

The application of evolutionary thinking to the study of cancer initiation, progression, heterogeneity, and resistance has produced many successful studies. However, several aspects of cancer remain underrepresented in the evolutionary literature and could benefit tremendously from more intensive quantitative investigations. The putative cancer stem cells and their dynamics during cancer progression and therapy, for example, have not received much attention. This fact may in part be due to the relative novelty of experimental studies of cancer stem cells and the comparatively small amount of biological information available to modelers; however, quantitative and evolutionary approaches to cancer stem cells will be necessary for driving the investigations proving (or disproving) their existence, their mechanisms of treatment insensitivity, and related aspects. Similarly, a full understanding of resistance from an evolutionary viewpoint still requires considerable work. Many cancers relapse despite therapy shown to be effective *in vitro*, and the causes of refractory disease remain to be identified in many cases. Pharmacological modeling such as the investigation of pharmacokinetics and pharmacodynamics in combination with the emergence of resistance similarly requires further input from evolutionary theorists. The questions about the optimum dosing strategies that utilize small therapeutic windows need to be answered with sophisticated mathematical models that push the field beyond the current state.

The main barriers to theoretical investigations of cancer remain a lack of generally accessible and quality-controlled databases of treatment response data, mutation status of cancers for resistance investigations, heterogeneity information in the form of genomic data such as SNP arrays, gene expression data and sequence information, and other data. Such data should not only provide spatial, but also temporal information about tumors. The development of inexpensive, high-throughput single-cell assays, as well as next-generation sequencing methods, will be beneficial for theoretical endeavors. Furthermore, most evolutionary parameters such as mutation rates, fitness landscapes of neoplasms, and population sizes and structures are virtually unknown. These parameters can be used to validate evolutionary models; for example, the predictions of the Luria–Delbrück distribution of resistance mutations pre-existing to therapy (Skipper 1983; Zheng 1999; Sarkar 1991; Angerer 2001; Frank 2003; Dewanji et al. 2005; Iwasa et al. 2006; Haeno et al. 2007; Komarova et al. 2007; Zheng 2008) should be tested in cell line experiments that can directly assay the frequency of apoptosis as well as the growth and mutation rates of cells. Such studies of experimental evolution remain rare but would provide valuable information to modelers. Once more data are accessible to theoreticians, the evolution of the field will be accelerated. Interdisciplinary investigations are furthermore hindered by the complications of securing funding. Most major medical funding sources prefer traditional molecular biology or biochemical studies to mathematical investigations, and the field would benefit tremendously from a funding source for interdisciplinary work. Also, the training of the next generation of evolutionary modelers of cancer requires the design of a useful curriculum, since the sheer amount of knowledge necessary (cancer biology, evolution, applied mathematics, bioinformatics, and statistics) renders most study courses superficial. Along the same line, evolutionary training of oncologists and clinicians would be beneficial for the understanding of cancer.

In closing, we believe that the application of evolutionary modeling to cancer will continue to increase our understanding of neoplasms and will eventually contribute to the design of systems biology-based strategies to detect and manage the disease.

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Conflicts of Interest

The authors declare no conflicts of interest.

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