Cancer Prevention Strategies That Address the Evolutionary Dynamics of Neoplastic Cells: Simulating Benign Cell Boosters and Selection for Chemosensitivity

Carlo C. Maley,[1,2] Brian J. Reid,[1,2,3,4] and Stephanie Forrest[5]

Divisions of [1]Human Biology and [2]Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington; Departments of [3]Medicine and [4]Genome Sciences, University of Washington, Seattle, Washington; and [5]Department of Computer Science, University of New Mexico, Albuquerque, New Mexico

Abstract

Cells in neoplasms evolve by natural selection. Traditional cytotoxic chemotherapies add further selection pressure to the evolution of neoplastic cells, thereby selecting for cells resistant to the therapies. An alternative proposal is a benign cell booster. Rather than trying to kill the highly dysplastic or malignant cells directly, a benign cell booster increases the fitness of the more benign cells, which may be either normal or benign clones, so that they may outcompete more advanced or malignant cells in a neoplasm. *In silico* simulations of benign cell boosters in neoplasms with evolving clones show benign cell boosters to be effective at destroying advanced or malignant cells and preventing relapse even when applied late in progression. These results are conditional on the benign cell boosters giving a competitive advantage to the benign cells in the neoplasm. Furthermore, the benign cell boosters must be applied over a long period of time in order for the benign cells to drive the dysplastic cells to extinction or near extinction. Most importantly, benign cell boosters based on this strategy must target a characteristic of the benign cells that is causally related to the benign state to avoid relapse. Another promising strategy is to boost cells that are sensitive to a cytotoxin, thereby selecting for chemosensitive cells, and then apply the toxin. Effective therapeutic and prevention strategies will have to alter the competitive dynamics of a neoplasm to counter progression toward invasion, metastasis, and death. (Cancer Epidemiol Biomarkers Prev 2004;13(8):1375–84)

Introduction

Curing cancer and preventing cancer are largely evolutionary problems (1, 2). Neoplasms are made up of heterogeneous populations of mutant cells (3-7) undergoing constant turnover (8-10). These cells compete for resources in and around the neoplasm. Any mutation that allows a cell to reproduce more rapidly or avoid apoptosis will tend to spread in the population of neoplastic cells. This is true both during progression and during treatment. Any therapy that kills cancer cells will also select for cells that are resistant to that therapy. The traditional therapeutic approach biases the clonal competition in a neoplasm by trying to kill the quickly proliferating cells or triggering differentiation (11-13) or senescence (14, 15). An alternative is to develop strategies to boost relatively benign cells, giving them a competitive advantage over the more dysplastic cells and allowing competition to select against dysplastic cells. In short, we propose to select for a benign neoplasm by increasing the relative fitness of the benign cells rather than by weeding out the dysplastic cells. The relative fitness of benign cells might be increased by increasing the proliferation rate, decreasing the rate of apoptosis and other forms of cell mortality, or boosting the capacity of the benign cell to compete for space and nutrients. We call such therapies "benign cell boosters". Here, we focus on benign cell boosters that increase the proliferation rate of benign cells, although successful benign cell booster therapies may have to modulate multiple characteristics of the benign cells to outcompete the more dysplastic cells in a neoplasm. We also consider therapies that select for chemosensitive cells prior to the application of a cytotoxin.

We have developed a set of models to simulate the evolutionary dynamics within a neoplasm (16). We use these simulations to explore ideas for chemotherapies and chemoprevention that directly address the evolutionary nature of cancer. Such simulations support the search for a mechanism that could produce the desired effects (4, 17-21). The models are an initial step in the development of a novel therapy that may stimulate experiments in cell cultures and animal models.

Benign Cell Boosters. Current therapies select for resistance and thus breed their own failure (22-25). If we could select for a benign neoplasm, even at the cost of increasing its size, then we could potentially cure patients through follow-up surgery. This form of therapy has three attractive characteristics. First, it should not

select for resistance. Because the booster would benefit cells, any cell that is resistant to the therapy would be at a competitive disadvantage relative to the sensitive cells. Second, benign cell boosters could be relatively nontoxic, because they would not need to directly kill or damage any cells. Third, they could be effective late in progression, even after the genomes of the neoplastic cells have become unstable. A benign cell booster should benefit both the benign cells that already exist in or around the neoplasm and any dysplastic cells that happen to mutate into a more benign state.

Materials and Methods

The Model. We have developed a computational simulation that includes the essential evolutionary dynamics found in a neoplasm (16). A mathematical description of models such as these is generally intractable. We have therefore used *in silico* simulation experiments to test our hypotheses. We simulated a population of neoplastic stem cells. Each stem cell was explicitly represented in the model. This allowed us to represent the heterogeneity within a neoplasm by simulating the processes of mutation, mitosis, and apoptosis in every cell. The cells were distributed on a two-dimensional surface wrapped into the shape of a column to represent a Barrett's esophagus neoplasm (26), although a three-dimensional mass can also be simulat-

ed. The model itself is generic, and its parameters were configured for the following experiments as described in Table 1.

The state of a cell was represented by a diploid set of loci and an age in time steps. A time step represented ~12 hours. The simulation began at a point just after initiation when all cells are neoplastic but had no other genetic lesions. The cells divided on average once every 4 days, with a SD of 1 day (10, 27, 28). The number of genes that must be mutated to produce cancer in most tissues is unknown but is commonly estimated to require 3 to 12 rate-limiting steps (29-31). There were four different types of loci represented in a simulated cell: selective loci with dominant mutations (e.g., oncogenes or haploinsufficient tumor suppressor genes, usually 3), selective loci with recessive mutations (e.g., tumor suppressor genes, usually 2), a mutator locus (e.g., a DNA repair gene), and a variable number of drug resistance loci (from 1 to 6), as depicted in Fig. 1. If both alleles of a recessive locus acquired mutations, the cell gained a selective advantage over its competitors. The mutation of a single allele in a dominant locus was sufficient to confer a selective advantage on the cell. The selective advantage due to a lesion in a locus (dominant or recessive) was implemented as a cumulative decrease in the average generation time of the cell by 12 hours (one-time step) per locus. When a stem cell divided, one daughter cell remained in the tissue and the other either displaced a neighbor or died, with 50% probability.

Table 1. The parameters of the model and the values that were used

Parameter	Values	Description
Dominant loci	0-6 loci (3 for therapy experiments)	A mutation in either allele of these loci reduces the average cell cycle time of the cell by one-time step.
Recessive loci	1-6 loci (2 for therapy experiments)	Both alleles of these loci must be mutated to reduce the average cell cycle time by one-time step.
Mutator loci	1 locus	No. of loci that when mutated increase the mutation rate of the cell.
Mutation rate	10^{-5}, 4×10^{-6}, 2×10^{-6}, 10^{-6}	The base mutation rate of the cells per locus per cell division.
Mutator factor	10 or 100 (100 in experiments)	Increase in the mutation rate for cells with mutator mutations (1 or 2 orders of magnitude).
Reversible mutations	Yes/no	Whether a mutant phenotype can be rescued by further mutations.
Time of chemotherapy	8,000, 16,000, or 24,000 time steps	The time step at which the cytotoxic therapy starts (~11, 22, and 33 years after initiation).
Follow-up time	3,600 time steps	5 years of follow-up after therapy to test the efficacy of the simulated treatment.
No. of drugs (no. of drug resistance loci)	1-6 drugs (loci)	No. of cytotoxic drugs requiring independent mutations for resistance. There is one resistance locus per drug.
Chemotherapy duration	One-time step	Cytotoxic therapies are applied in a single time step.
Chemotherapy efficacy	1	The probability a sensitive cell dies in one-time step in the presence of cytotoxins.
Time of benign booster	8,000, 16,000, or 24,000 time steps	Time at which the benign booster treatment begins.
Benign booster duration	3,600 time steps	The benign boosters are applied over the entire duration of the follow-up (5 years).
Benign target locus	Dominant, recessive, mutator, drug resistance	The locus used by the benign cell booster to distinguish benign (wild-type at locus) from nonbenign (mutated at locus) cells.
No. of cells in the neoplasm	4,096 cells	The simulated neoplasm consists of 4,096 cells in a 64 × 64 cell grid on the surface of a simulated tube.

NOTE: The model can simulate a variety of assumptions about the nature of progression and dysplasia.
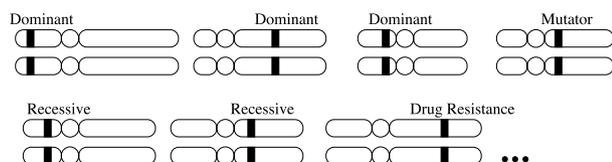
Figure 1. A schematic of the genome of a simulated cell. The placement of the loci on different chromosomes is arbitrary. All loci are effectively linked due to the asexual reproduction of neoplastic cells, but mutations affect each locus independently. In most runs of the model, each diploid cell had three loci in which a dominant mutation gave the cell a selective advantage by increasing its rate of mitosis. The cells also had two loci, which required recessive mutations in both alleles before the cell increased its rate of mitosis. Mutation of a single allele in the mutator locus increased the mutation rate of the cell, and a drug resistance locus conferred resistance to the drug associated with that locus.

All mutations were assumed to be passed on at mitosis, and DNA synthesis was assumed to be mitogenic, so that an increase in the rate of mitosis by definition increased the rate of mutations per unit of time. A mutation may be any form of genetic or epigenetic lesion that is heritable, including deletions, insertions, point mutations, methylation, gene conversions, amplifications, etc. Mutations were simulated with a Bernoulli process (biased coin flip), with a probability μ of each allele mutating per cell generation.[6] All the loci mutated independently, so there was no representation of linkage. In most runs of the model, the rate of allele mutation per cell per cell generation μ is 10^{-6}, which is ~1 order of magnitude higher than that observed *in vitro* (33-36) but was necessary due to computational time limitations. A parameter of the model determined if the mutations in the dominant, recessive, and mutator loci were reversible. This represented both back mutations and compensatory mutations elsewhere in the genome (37). Drug resistance was assumed to be reversible (38-42). We defined the latest dysplastic stage before invasion or metastasis to be a cell with mutations in all three dominant loci and both alleles of both recessive loci. We call this stage high-grade dysplasia (HGD). The mutator and drug resistance mutations were assumed to be dominant. Mutator mutations increased the mutation rate in the cell by a parameterized factor $f = 100$ (36, 43, 44).

For computational speed, we simulated a population of 4,096 cells. Simulations of 10^6 cells have been shown to be qualitatively similar to these smaller simulations (16). Like Barrett's esophagus neoplasms, the population of neoplastic cells did not grow over time (45). The results should be the same for a neoplasm that increases over time like a colonic adenoma, as long as there is a turnover in the cell population. The model most closely resembles premalignant neoplasms or tissues with high rates of turnover.

Experiments were carried out by starting the model just after the point of initiation, with no additional mutated loci in any of the cells. The model was run for 11, 22, or 33 years (8,000, 16,000, or 24,000 time steps). At that point, some form of therapy was applied to the model. The model was run for an additional 5 years (3,600 time steps) of follow-up and checked for the presence of HGD cells.

Modeling Cytotoxic Therapies. The simulated cytotoxic chemotherapies affected all cells in the neoplasm in the same way. A parameter determined the efficacy of the cytotoxic therapy, represented as the probability that the drug kills a sensitive cell in one-time step. In all the following results, the drugs always killed the sensitive cells in a single time step. A further parameter, $r = 1$ to 6, determined the number of independent drug resistance loci that would have to mutate to confer resistance to the multidrug cytotoxic cocktail. For simplicity, we think of this as applying r drugs with independent mechanisms for killing the cell. We did not explicitly simulate multidrug resistance loci, although such a locus would make multiple drugs act as a single independent drug in the model. Cytotoxic therapies were applied in a pulse of a single time step.

We varied the mutation rate $\mu = 1 \times 10^{-6}$, 2×10^{-6}, 4×10^{-6}, or 10^{-5}. The model was run at least 2,500 times for each parameter setting and as many as 169,200 times for the conditions that had a low probability of producing HGD. It was necessary to run the model many times because only the runs that produced HGD by the time of the therapy could be used to calculate the efficacy of the therapies. Higher mutation rates might be used to raise the probability of producing HGD, but high mutation rates would also bias the outcome of the therapies.

Modeling Benign Cell Boosters. We simulated benign cell booster therapies by decreasing the generation time of "benign" cells that were sensitive to the booster drug. A cell was sensitive to the booster ("benign") if it did not have a mutant allele at the locus targeted by the booster. We simulated four different kinds of benign cell boosters: those that targeted a particular selective locus lacking either dominant or recessive mutant alleles, the mutator locus, or a cytotoxic drug resistance (and thus irrelevant) locus. If the target locus was one of the selective loci with recessive alleles, then a cell was sensitive to the booster if at least one allele was still wild-type. Other target loci, with dominant alleles, required two wild-type alleles to be sensitive. In the presence of the booster, sensitive cells divided on average one-time step (12 hours) faster than HGD cells. Benign cell boosters were applied to the system for the entire 5 simulated years of follow-up to facilitate cellular competition. As an experimental control, an additional locus was added to the simulated genome of a cell for resistance to the benign cell booster. If either allele of that locus was mutated, then the cell received no benefit from the booster. Because this would be a disadvantage for the cell, there was selection pressure against mutations in this locus in the presence of a benign cell booster. The model was run between 2,500 and 89,200 times for each parameter setting.

---

[6] We abandoned an approximation used in the earlier version of the model (16) and directly calculated the interarrival time, i.e., the number of mitoses, until the next mutation as a geometric random variable (32).

Results

We explored a range of parameters for three different therapy strategies: cytotoxic chemotherapies, benign cell boosters, and a combination of the two. In all cases, we collected data for early (8,000 time steps ≈ 11 years of simulated time), intermediate (16,000 time steps ≈ 22 years of simulated time), and late (24,000 time steps ≈ 33 years of simulated time) application of the therapies relative to the initiation of the neoplasm. This approximates the time scale of progression in human neoplasms (45-47). The probability of the simulated neoplastic tissue developing HGD in the absence of therapy is shown in Table 2. A ''cure'' was defined by the presence of HGD cells at the time of therapy and the absence of HGD cells 5 simulated years after the therapy. Minimal residual disease was defined as a frequency of <1% HGD cells in the neoplasm 5 years after therapy.

Cytotoxic Therapies. Adding more drugs that require more mutations for resistance to the cytotoxic therapy dramatically improves the performance of the therapy. However, the later the therapy is applied after initiation and the higher the mutation rate of the neoplastic cells, the lower the probability that the therapy will achieve a cure (Fig. 2).

Benign Cell Boosters. Unlike cytotoxic therapies, benign cell boosters were applied for the entire 5 simulated years of follow-up. Half a day of simulated time was not long enough for the benign (booster sensitive) cells to drive the HGD cells to extinction. Benign cell boosters often result in minimal residual disease (<1% HGD cells) rather than an outright cure. In Fig. 3, we have combined cures and minimal residual disease to quantify the effects of the boosters. A typical run of the model can be seen in Fig. 4.

Benign cell boosters were most effective when mutations in the loci they targeted could be rescued to restore the benign state. In this case, dysplastic cells might drive the benign cells to extinction, but some dysplastic cells would occasionally revert to the benign state and thereafter benefit from the benign cell booster. When the phenotypes of the loci could be rescued by further mutations, targeting the wild-type phenotype of dominant or recessive loci with a benign cell booster was almost guaranteed to affect a cure or minimal residual disease, even very late in progression and regardless of the mutation rate (Fig. 3). If the lesions in loci could not be rescued by further mutations, boosting cells that had not suffered a recessive mutation worked best. In this case, success of the benign cell booster declined with

higher mutation rates. Targeting the neutral or mutator loci was not particularly effective (Fig. 3). Mutations at neutral or mutator loci were not strictly necessary for producing HGD by definition, so selecting for the wild-type phenotypes did not prevent the development of HGD. We found that the only times that benign cell boosters failed were cases where either they targeted a mutation that was not directly involved in dysplasia or there were few to no benign cells left at the time of the application of the therapy such that the benign cells died before they could reestablish a competitive population. In these cases, by the start of the benign cell booster therapy, the HGD cells had already driven the benign cells to extinction or to such a small cell population that the few remaining benign cells happened to die off due to stochastic population fluctuations (genetic drift) before they could grow to a large enough population size to be buffered from those fluctuations.

We wondered if it was necessary to give the benign cells a competitive advantage over the HGD cells. If benign cells merely reproduced equally as fast as HGD cells, then mutations producing HGD would effectively be neutral. We call these ''weak benign cell boosters''. Weak boosters are clearly ineffectual, whether or not the targeted mutations are reversible (Fig. 5).

Benign cell boosters and cytotoxic therapies affect cures through different methods. We wondered if combining the forms of therapies might have complementary effects. We examined three possible combination therapies. We first tried the simultaneous application of a three-drug cytotoxic therapy and a benign cell booster that targeted a recessive locus. We simulated a sequential application, starting with a benign cell booster targeting a recessive locus and following it up, after 5 years, with the cytotoxic therapy. The intention was to let the benign cell booster reduce the HGD population to a few cells and try to kill off those cells with the cytotoxic therapy. Finally, we tried applying a benign cell booster therapy that, instead of boosting benign cells per se, boosted cells that were sensitive to the cytotoxic chemotherapy. We hoped to select for a neoplasm that would be exquisitely susceptible to the cytotoxins. We then applied the three-drug cytotoxic chemotherapy to eradicate the neoplasm. We called this strategy the ''sucker's gambit''. The results are shown in Fig. 6 and are compared with a cytotoxic therapy that requires three independent mutant loci for chemoresistance as well as a benign cell booster that targets a recessive locus in which lesions could be rescued by mutations that restored the benign state.

All the therapies work well early in progression. However, late in progression, the sucker's gambit

Table 2. The percent chance of developing high grade dysplasia

Time since initiation	Mutation rate (μ)			
	10^{-6}	2×10^{-6}	4×10^{-6}	10^{-5}
11 years (8,000 time steps)	0.07 (0.01)	2.25 (0.74)	15.00 (1.79)	58.50 (2.46)
22 years (16,000 time steps)	2.76 (0.33)	15.50 (1.81)	36.25 (2.40)	74.50 (2.18)
33 years (24,000 time steps)	7.72 (0.53)	17.25 (1.89)	44.75 (2.49)	89.00 (1.56)

NOTE: Results are based on a neoplasm of 4,096 cells with three dominant and two recessive mutations necessary for HGD, mutation rate μ per locus per cell division, reversible mutations, and mutator phenotype increasing the mutation rate by a factor $f = 100$. The simulations are stochastic based on the use of a random number generator, and so there is variation in the results across runs. SEs calculated from a Bernoulli process are in parentheses. There were at least 400 runs for each parameter setting.
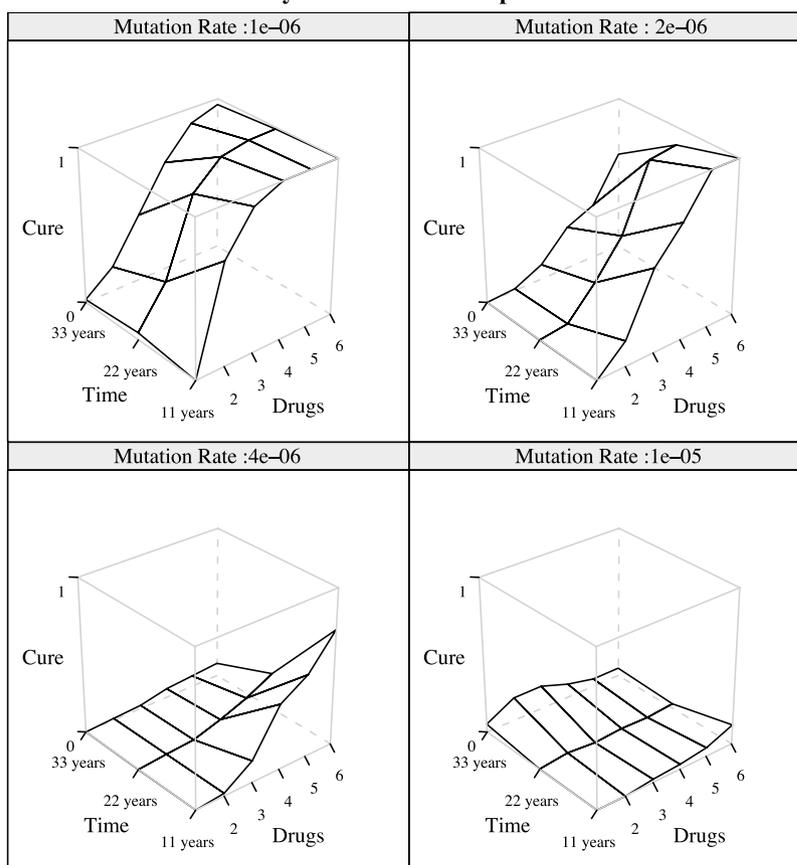
Cytotoxic Chemotherapies

Figure 2. The probability of achieving a cure with a cocktail of cytotoxic drugs. The base mutation rate of the cells in the neoplasm is lowest (10^{-6}) in the *upper left-hand panel* and greatest (10^{-5}) in the *lower right-hand panel*. The number of drugs requiring independent mutations for resistance and the time since initiation of the neoplasm at which the therapy is applied varied within each panel. Time since initiation is measured in simulated years since the initiation of the neoplasm.

removes all of the HGD cells significantly more often than any one therapy alone (Fisher's exact test $P < 0.001$) as well as the other combination therapies that we examined (Fisher's exact test $P < 0.001$). The benign cell booster targeting a recessive locus seems to perform similarly to the three–cytotoxic drug cocktail. However, this is slightly misleading because the cases that are not absolutely cured by the benign cell boosters are generally reduced to minimal residual disease, as shown earlier in Fig. 3.

Discussion

Benign cell boosters take advantage of the evolutionary dynamics within a neoplasm to select for a benign neoplasm. We have reversed the traditional strategy of chemotherapy by helping cells to survive or proliferate rather than destroying them. The simulations show that boosting benign cells is a promising approach even late in progression when traditional therapies tend to fail. They also suggest a powerful way to combine cytotoxins with boosters by first selecting for chemosensitivity and using a traditional cocktail of cytotoxins. The results further support Goldie and Coldman's (48) contention that the earlier the application of a cytotoxic therapy, the better. They also support the standard practice of using multidrug cocktails (49-51).

Our approach to chemoprevention is similar in spirit to the use of probiotics to drive out pathogenic intestinal flora by introducing benign intestinal flora with a superior relative fitness (52-54). Similarly, anaerobic bacteria have been used to outcompete cancer cells in the poorly vascularized areas of a neoplasm (55, 56).

Gatenby et al. have developed similar models of carcinogenesis and invasion based on clonal competition (21, 57, 58). Their models support a variety of therapeutic strategies including decreasing the carrying capacity of the environment for tumor cells, increasing the competitive effects of normal cells on tumor cells, decreasing the competitive effects of tumor cells on normal cells (21), and decreasing the pH levels in the tumor environment (57). We are suggesting an additional strategy, which in their models might be represented by increasing the net growth rate of the normal cells.

When benign cell boosters fail, it is because there are no benign cells left to boost. If phenotypes can be rescued by further mutations to produce the benign state, this is not a problem. Work in experimental evolution suggests that mutant phenotypes are typically reversible through a compensating mutation (37). Mutations that compensate for a deleterious mutation in bacteriophage φ6 seem to be more common than back mutations (59). Similarly, *Escherichia coli* that suffer a fitness reduction from carrying the pACYC184 resistance plasmid in the absence of antibiotic have been shown to evolve a
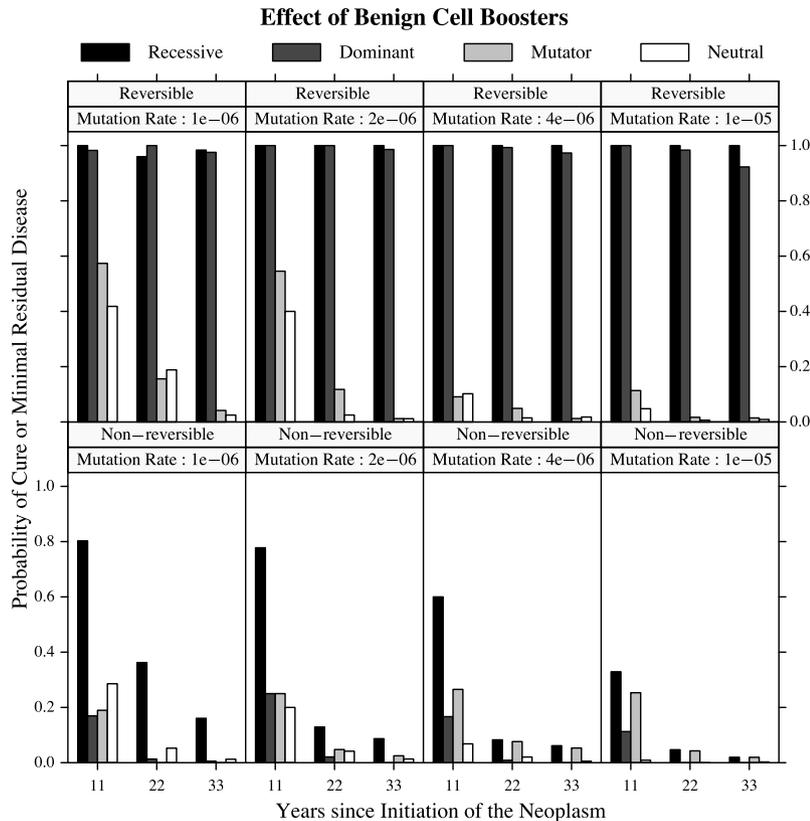
Figure 3. The effect of benign cell booster therapies at 11, 22, or 33 years after the initiation of the neoplasm for mutation rates of $\mu = 10^{-6}$, 2×10^{-6}, 4×10^{-6}, and 10^{-5} per allele per cell division. The different types of benign cell boosters are distinguished by the type of targeted locus that had to have a wild-type allele in order for the cell to receive the benefit of the booster. *Upper panels,* the phenotypes of the loci could be rescued by mutations to produce the wild-type phenotype either by a back mutation or by a compensating mutation. *Lower panels,* mutant phenotypes could not be rescued. The neutral (cytotoxic resistance) locus could be rescued in either case, but its state had no effect on the reproduction or survival of the cells in the absence of the booster therapy.

compensating mutation that reverses the fitness effect of the plasmid rather than removing the plasmid (60). If mutant phenotypes cannot be rescued to produce the wild-type phenotype, it might be possible to inoculate the neoplasm with benign cells, much as Dang et al. (56) inoculated their neoplasms with bacteria. However, this could be impractical for metastatic cancers.

In the model, benign cell boosters tend to result in minimal residual disease, with a constant but low-frequency appearance of HGD cells. This is probably not due to a failure in the competitive dynamics but rather a constant introduction of new HGD cells that are subsequently driven extinct. There are no selective forces in the model that prevent the "benign" cells from developing mutations in all the other carcinogenic loci that are not targeted by the benign cell booster. Thus, there may be some cells in the neoplasm that are only one hit away from HGD. If the neoplasm cannot be removed physically, benign cell boosters might have to be used indefinitely to restrain the emergence of new HGD cells.

We studied only one mechanism of boosting benign cells, by increasing their rate of reproduction. Alternatively, one might look for a way to reduce their rate of apoptosis below that of the HGD cells or increase the ability of the benign cells to compete for space and nutrients. In any case, proliferating benign cells in a neoplasm might increase the total mass of the neoplasm. We expect this to be an acceptable tradeoff for evolving a benign neoplasm, which often may be surgically removed. In some cases, such as Barrett's esophagus, the total premalignant neoplasm size remains relatively constant over time even in the presence of rampant neoplastic cell proliferation (45). In such tissues, benign cell boosters might not affect the size of the neoplasm.

The *in silico* explorations highlight several qualifications to our encouraging results. A benign cell booster must target a characteristic of a cell that is causally related to the benign state. If the target were only correlated with the benign state, like the cytotoxic sensitivity alleles, then we would select for dysplastic cells that also carried the target of the benign cell booster. Furthermore, benign cell boosters would probably have to be applied to a neoplasm over a long period of time to allow intraneoplasm competition to drive the dysplastic cells to extinction and to prevent the reemergence of HGD clones. This lengthy regime is not unique to benign cell boosters. Tamoxifen is used to treat breast cancer over a course of ≥5 years (61). It is our hope that non-toxic boosters would make extended applications of the therapy feasible. Benign cell boosters are unlikely to work for metastatic disease because the malignant cells are no longer spatially constrained, although the sucker's gambit might still be used to select for malignant cells that are primed to take up a cytotoxin.

Our simulation results are based on a simplified representation of neoplastic evolution and chemotherapy. The next step would be to translate these ideas into a cell culture or animal model. In doing so, it will be important to bear in mind some caveats. We have worked with small cell populations with only 4,096 cells and relatively high mutation rates of 10^{-6} per locus per generation.

Lower mutation rates are computationally intractable because the production of HGD would either require many more runs or many more cells and much more computation. The effects of a larger cell population might be offset by a lower mutation rate. However, the estimates of the model are probably conservative because small populations are more vulnerable to genetic drift, which can interfere with natural selection. The only interaction we have represented between cells is competition for space, with no cytokines or contact inhibition. In addition, we did not explicitly represent the potential neoplasm suppressor effects of tissue architecture (1), differentiation, or apoptosis, all of which are subsumed in the displacement of a cell by the reproduction of a neighbor. Nor did we explicitly represent neoangiogenesis, invasion, or metastasis. Instead, the various phenotypes necessary for cancer have been abstracted into the requirement of a specific number of dominant and recessive mutations before a neoplastic tissue develops HGD. The representation of chemotherapy was also highly simplified. There were no variations in drug dosage experienced by the cells. In reality, the cells on the interior of a solid tumor are generally less exposed to chemotherapies and experience different selective pressures than the cells on the surface of a tumor. Our estimates of the efficacy of cytotoxic therapies are probably overly optimistic because we did not simulate the phenomenon of single mutations that confer multidrug resistance (22). These elaborations may be introduced into the model if they become necessary to test future hypotheses for the development of a benign cell booster.

We represented resistance to chemotherapies as a characteristic of a cell. If a dysplastic cell could release an extracellular factor to interfere with the benign cell booster, then dysplastic cells could evolve a form of resistance to benign cell boosters. However, we would expect selection pressure on the benign cells to interfere with the factor released by the dysplastic cells and vice versa. A genetic arms race would ensue.

Developing a Benign Cell Booster

The most important caveat to these results is that we do not yet know how to make a benign cell booster. A benign cell booster would alter the environment of the neoplasm so as to give benign cells a competitive advantage over dysplastic cells. Protective factors such as changes in diet, ingestion of selenium, cyclooxygenase-2 inhibitors, or use of nosteroidal anti-inflammatory drugs might act through this mechanism (62-64). It is possible that benign cell boosters already exist in various drug screens but have been missed because they had no therapeutic effect on malignant cells. To discover a benign cell booster, we will need to screen for effects on clonal competition.
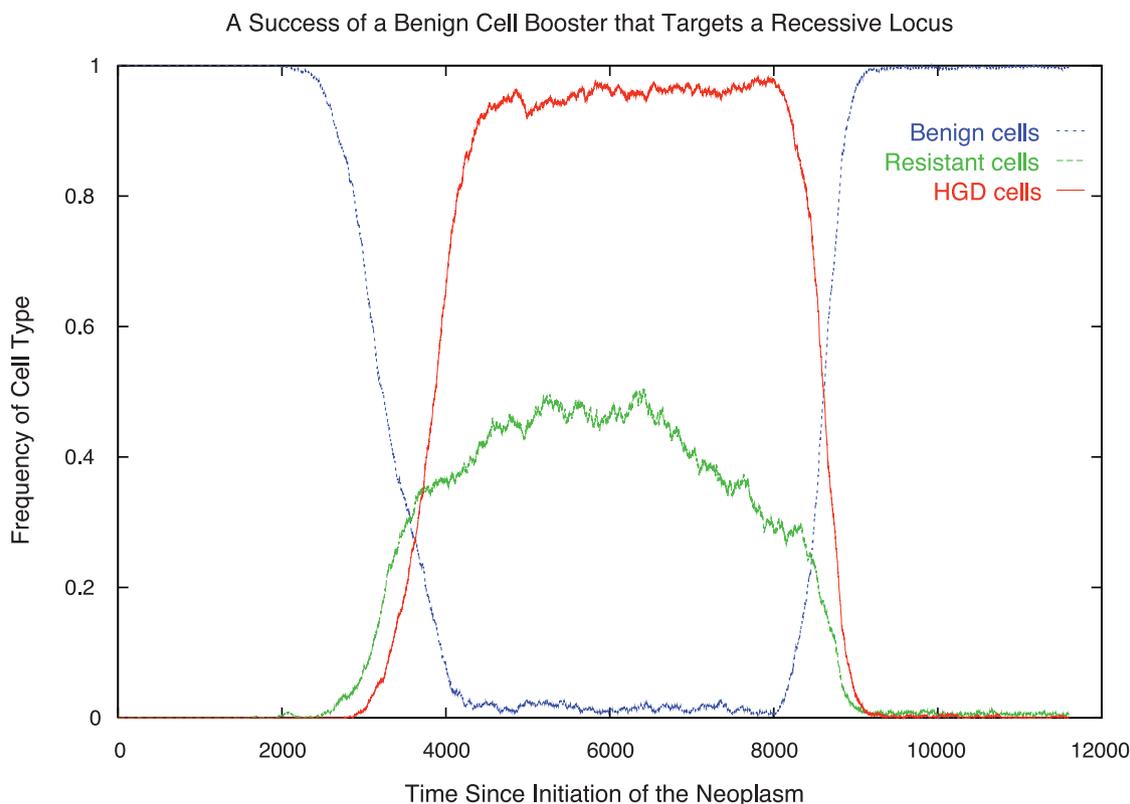

Figure 4. The frequency of the HGD cells are plotted (*red*) and can be seen to take over the neoplasm around time step 4,000. The ''benign'' cells with at least one wild-type allele in the targeted recessive locus are driven near to extinction. However, mutations at the loci can be rescued by further mutations, and so when the benign cell booster is applied at time step 8,000, benign cells emerge that outcompete the HGD cells and any cells resistant to the benign cell booster.

Weak Benign Cell Boosters
with Non−Reversible Mutations
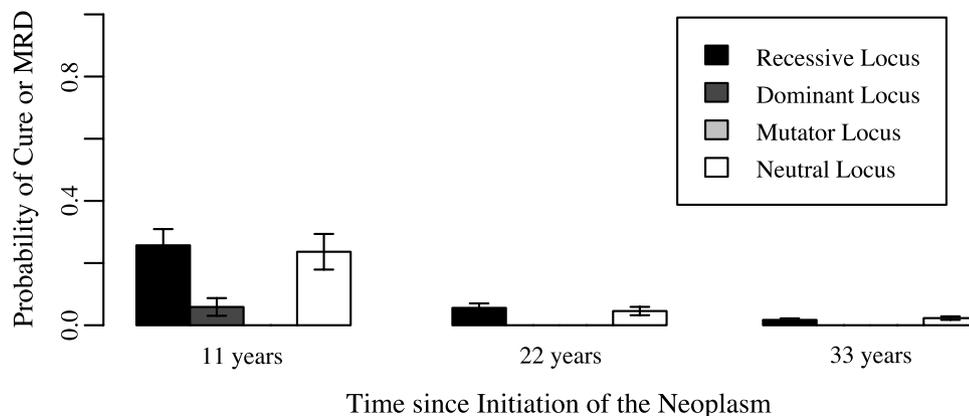
Weak Benign Cell Boosters
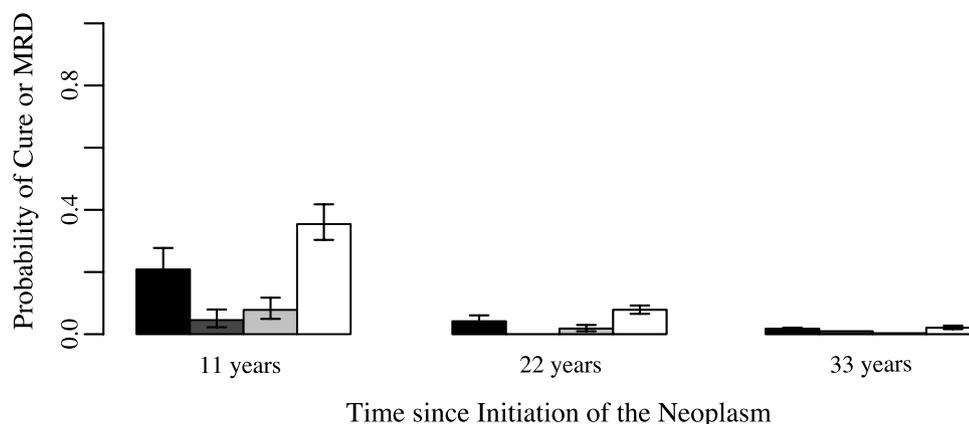## with Reversible Mutations

Figure 5. Cure and minimal residual disease (*MRD*) probabilities for ''weak'' benign cell boosters that only allow the benign cells to reproduce at the same but not a greater rate than the HGD cells. *Large SE bars* on therapies applied at 11 years are due to the fact that few cancers evolve in only 11 simulated years in 4,096 cells and so the estimates are based on only a few data points.

Cell culture and animal model experiments to find a benign cell booster would be similar to one another. They would require two distinguishable clones, one more dysplastic than the other. The two clones would be mixed and introduced to the culture or animal model (65-67). In the absence of a treatment, we predict that the dysplastic clone would outcompete the other clone. Benign cell boosters could be identified among those substances or manipulations that produce a pure population of the more benign clone. If such a manipulation is successful, the experiment could be repeated across other cell lines and induced neoplasms in animal models.

Work in the Shionogi, LNCaP, and LuCaP 23.12 rat prostate neoplasm models showed that intermittent withdrawal of androgens causes a cycling of prostate-specific androgen (PSA) levels and delays androgen-independent neoplasm growth (68-70). Cycling in PSA levels may indicate cellular competition within the neoplasm between androgen-sensitive and androgen-independent clones. In the presence of androgens, the androgen-sensitive clone may have a competitive advan-

tage over the androgen-independent clone. When the androgens are removed, the sensitive cells may begin to die, lowering PSA levels, giving androgen independent clones a competitive advantage, and eventually restoring PSA levels. In cases where the only androgen-sensitive cells remaining are benign, the withdrawal of antiandrogen therapies may allow the benign cells to outcompete androgen-independent malignant clones and thus explain the observation of regression after antiandrogen withdrawal (71, 72). However, it is unclear if the cycling in PSA levels derive from cycling in clone population sizes. Although the Shionogi and LuCaP 23.12 models show apoptosis, the LNCaP model does not, and so Sato et al. (69) suggest that they may be measuring changes in the expression of PSA in a static cell population rather than clonal competition between androgen-sensitive and androgen-independent cells.

It may be possible to generalize the example of intermittent androgen withdrawal. By supplying a nutrient or substrate to a neoplasm, one could select for cells that take up that substrate. Then, withdrawing the substrate could cause a temporary regression in the neoplasm. It
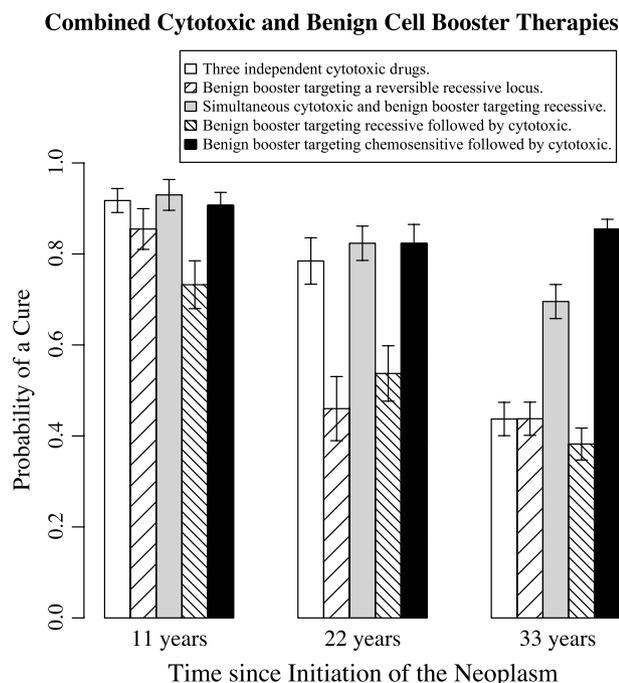
Combined Cytotoxic and Benign Cell Booster Therapies

Figure 6. A comparison of five different simulated therapies early, intermediate, and late in progression for mutation rate $\mu = 10^{-6}$ per allele per cell division. Minimal residual disease has been excluded from these results and so the cure rates for the benign therapies seem lower than those shown in Fig. 3.

might be possible repeatedly to select the cells in the neoplasm for an addiction to some substrate and withdraw that critical substrate, knocking the neoplasm back and delaying progression.

Even if designing a therapy to boost benign cells proved infeasible, without also boosting the dysplastic cells, we could still play the sucker's gambit. A sucker's gambit experiment would add a nutrient that mimics a cytotoxin to a culture or neoplasm. Cells that could take up the nutrient would outcompete cells that could not take up the nutrient, resulting in a neoplasm primed to take up the cytotoxin. After cultivating the cells in this enriched environment, the cytotoxin would be applied and the effectiveness of the toxin would be measured. We predict that the cytotoxin would be more effective on cells bred in the presence of the nutrient mimic than cells bred in an unaltered environment or in an environment with a nutrient that did not mimic the cytotoxin. In this way, a sensitive cell booster could be used as an adjuvant preparatory therapy prior to the application of a traditional chemotherapy.

An abstract model such as this one can be used to explore complex theories for which intuition is only a weak guide. Simulations can identify possibilities and rule out some potential avenues of research. In this case, the simulation suggests that a benign cell booster may work even late in progression and the sucker's gambit should be an effective way to combine traditional cytotoxic therapies with an evolutionary strategy of selecting for chemosensitivity. The model suggests that selection on loci directly related to carcinogenesis should

be more effective than selection on loci involved indirectly in carcinogenesis through genetic instability. It also cautions us that a benign cell booster would have to give benign cells an appreciable competitive advantage over dysplastic cells. In this way, our modeling approach helps to refine which experiments are worth pursuing in the laboratory and eventually in humans.

Conclusions

We must look for ways of disrupting the evolutionary dynamics of progression. Promising results can be achieved *in silico*. Here, we explored the use of clonal competition within a neoplasm to select for a more benign neoplasm. Alternatively, the efficacy of traditional therapies might be amplified by first selecting for sensitivity to cytotoxins. We predict that both traditional therapies and benign cell boosters will be more effective as chemoprevention strategies rather than chemotherapies, because neoplasms early in progression are less likely to harbor resistant clones than neoplasms late in progression. Taking advantage of this will depend on the early detection of precancerous neoplasms and better prediction of which precancerous neoplasms are likely to progress.

References

1. Cairns J. Mutation selection and the natural history of cancer. Nature 1975;255:197-200.
2. Nowell PC. The clonal evolution of tumor cell populations. Science 1976;194:23-8.
3. Barrett MT, Sanchez CA, Prevo LJ, et al. Evolution of neoplastic cell lineages in Barrett oesophagus. Nat Genet 1999;22:106-9.
4. Gonzalez-Garcia I, Sole RV, Costa J. Metapopulation dynamics and spatial heterogeneity in cancer. Proc Natl Acad Sci USA 2002;99: 13085-9.
5. Fujii H, Marsh C, Cairns P, Sidransky D, Gabrielson E. Genetic divergence in the clonal evolution of breast cancer. Cancer Res 1996; 56:1493-7.
6. Califano J, van der Riet P, Westra W, et al. Genetic progression model for head and neck cancer: implications for field cancerization. Cancer Res 1996;56:2488-92.
7. Coons SW, Johnson PC, Shapiro JR. Cytogenetic and flow cytometry DNA analysis of regional heterogeneity in a low grade human glioma. Cancer Res 1995;55:1569-77.
8. Raza A. Consilience across evolving dysplasias affecting myeloid, cervical, esophageal, gastric and liver cells: common themes and emerging patterns. Leuk Res 2000;24:63-72.
9. Preston-Martin S, Pike MC, Ross RK, Jones PA, Henderson BE. Increased cell division as a cause of human cancer. Cancer Res 1990; 50:7415-21.
10. Wright NA, Alison M. The biology of epithelial cell populations. Oxford: Clarendon Press; 1984.
11. Scott RE. Differentiation, differentiation/gene therapy and cancer. Pharmacol Ther 1996;73:51-65.
12. Leszczyniecka M, Roberts T, Dent P, Grant S, Fisher PB. Differentiation therapy of human cancer: basic science and clinical applications. Pharmacol Ther 2001;90:105-6.
13. Hansen LA, Sigman CC, Andreola F, Ross SA, Kelloff GJ, De Luca LM. Retinoids in chemoprevention and differentiation therapy. Carcinogenesis 2000;21:1271-9.
14. Roninson IB. Tumor cell senescence in cancer treatment. Cancer Res 2003;63:2705-15.

15. Saretzki G. Telomerase inhibition as cancer therapy. Cancer Lett 2003;194:209-19.
16. Maley CC, Forrest S. Exploring the relationship between neutral and selective mutations in cancer. Artif Life 2000;6:325-45.
17. Gardner SN. Cell cycle phase-specific chemotherapy: computational methods for guiding treatment. Cell Cycle 2002;1:369-74.
18. Abkowitz JL, Catlin SN, Guttorp P. Strategies for hematopoietic stem cell gene therapy: insights from computer simulation studies. Blood 1997;89:3192-8.
19. Boman BM, Fields JZ, Bonham-Carter O, Runquist OA. Computer modeling implicates stem cell overproduction in colon cancer initiation. Cancer Res 2001;61:8408-11.
20. Shackney SE, Smith CA, Miller BW, et al. Model for the genetic evolution of human solid tumors. Cancer Res 1989;49:3344-54.
21. Gatenby RA, Vincent TL. Application of quantitative models from population biology and evolutionary game theory to tumor therapeutic strategies. Mol Cancer Ther 2003;2:919-27.
22. Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer 2002;2:48-58.
23. Greenwood E. Drug resistance: resisting arrest. Nat Rev Cancer 2002; 2:406.
24. Krishnadath KK, Wang KK, Taniguchi K, et al. p53 mutations in Barrett's esophagus predict poor response to photodynamic therapy. Gastroenterology 2001;120:A-413.
25. Gorre ME, Mohammed M, Ellwood K, et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 2001;293:876-80.
26. Paulson TG, Reid BJ. Barrett's esophagus: molecular biology and pathology. In: Abbruzzese JL, Evans DB, Willett C, Feoglio-Preiser C, editors. Principles and practice of gastrointestinal oncology. Oxford (United Kingdom): Oxford University Press; 2003.
27. Herbst JJ, Berenson MM, McCloskey DW, Wiser WC. Cell proliferation in esophageal columnar epithelium (Barrett's esophagus). Gastroenterology 1978;75:683-7.
28. Madara JL. Epithelia: biologic principles of organization. In: Yamada T, editor. Textbook of gastroenterology. 2nd ed. Philadelphia: J.B. Lippincott Co.; 1995. p. 237-57.
29. Renan MJ. How many mutations are required for tumorigenesis? Implications from human cancer data. Mol Carcinog 1993;7:139-46.
30. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100: 57-70.
31. Vogelstein B, Kinzler KW. The multistep nature of cancer. Trends Genet 1993;9:138-41.
32. Drake AW. Fundamentals of applied probability theory. New York (NY): McGraw-Hill, Inc.; 1967.
33. Oller AR, Rastogi P, Morgenthaler S, Thilly WG. A statistical model to estimate variance in long term-low dose mutation assays: testing of the model in a human lymphoblastoid mutation assay. Mutat Res 1989;216:149-61.
34. Monnat RJ Jr. Molecular analysis of spontaneous hypoxanthine phosphoribosyltransferase, mutations in thioguanine-resistant HL-60 human leukemia cells. Cancer Res 1989;49:81-7.
35. Fukuchi K, Martin GM, Monnat RJ Jr. Mutator phenotype of Werner syndrome is characterized by extensive deletions. Proc Natl Acad Sci USA 1989;86:5893-7.
36. Seshadri R, Kutlaca RJ, Trainor K, Matthews C, Morley AA. Mutation rate of normal and malignant human lymphocytes. Cancer Res 1987; 47:407-9.
37. Teotonio H, Rose MR. Reverse evolution. Evolution 2001;55:653-60.
38. Tsuruo T. Molecular cancer therapeutics: recent progress and targets in drug resistance. Int Med 2003;42:237-43.
39. Frenkel GD, Caffery PB. A prevention strategy for circumventing drug resistance in cancer chemotherapy. Curr Pharm Des 2001;7: 1595-614.
40. Kobayashi H, Takemura Y, Miyachi H. Novel approaches to reversing anti-cancer drug resistance using gene-specific therapeutics. Hum Cell 2001;14:172-84.
41. Naito S, Koga H, Yokomizo A, et al. Molecular analysis of mechanisms regulating drug sensitivity and the development of new chemotherapy strategies for genitourinary carcinomas. World J Surg 2000;24:1183-6.
42. El-Deiry WS. Role of oncogenes in resistance and killing in cancer therapeutic agents. Curr Opin Oncol 1997;9:79-87.
43. Simpson AJG. The natural somatic mutation frequency and human carcinogenesis. Adv Cancer Res 1997;71:209-39.
44. Boyer JC, Umar A, Risinger JI, et al. Microsatellite instability, mismatch repair deficiency, and genetic defects in human cancer cell lines. Cancer Res 1995;55:6063-70.
45. Cameron AJ, Lomboy CT. Barrett's esophagus: age, prevalence, and extent of columnar epithelium. Gastroenterology 1992;103: 1241-5.
46. Gryfe R, Swallow C, Bapat B, Redston M, Gallinger S, Couture J. Molecular biology of colorectal cancer. Curr Probl Cancer 1997;21: 233-300.
47. Kelloff GJ, Boone CW, Malone WF, Steele VE, Doody LA. Development of chemopreventive agents for bladder cancer. J Cell Biochem 1992;161:1-12.
48. Goldie G, Coldman A. The genetic origin of drug resistance in neoplasms: implications for systemic therapy. Cancer Res 1984;44: 3643-53.
49. DeVita VT Jr, Chu E. Physicians' cancer chemotherapy drug manual. Boston: Jones and Bartlett; 2003.
50. Parimoo D, Raghavan D. Progress in the management of metastatic bladder cancer. Cancer Control 2000;7:347-56.
51. Hughes RS, Frenkel EP. The role of chemotherapy in head and neck cancer. Am J Clin Oncol 1997;20:449-61.
52. Marteau P, Seksik P, Jian R. Probiotics and health: new facts and ideas. Curr Opin Biotechnol 2002;13:496-9.
53. Tuohy KM, Probert HM, Smejkal CW, Gibson GR. Using probiotics and prebiotics to improve gut health. Drug Discov Today 2003;8: 692-700.
54. Rolfe RD. The role of probiotic cultures in the control of gastrointestinal health. J Nutr 2000;130:396S-402S.
55. Jain RK, Forbes NS. Can engineered bacteria help control cancer? Proc Natl Acad Sci USA 2001;98:14748-50.
56. Dang LH, Bettegowda C, Huso DL, Kinzler KW, Vogelstein B. Combination bacteriolytic therapy for the treatment of experimental tumors. Proc Natl Acad Sci USA 2001;98:15155-60.
57. Gatenby RA, Gawlinski ET. The glycolytic phenotype in carcinogenesis and tumor invasion: insights through mathematical models. Cancer Res 2003;63:3847-54.
58. Gatenby RA, Vincent TL. An evolutionary model of carcinogenesis. Cancer Res 2003;63:6212-20.
59. Burch CL, Chao L. Evolution by small steps and rugged landscapes in the RNA virus ϕ6. Genetics 1999;151:921-7.
60. Lenski RE, Simpson SC, Nguyen TT. Genetic analysis of a plasmid-encoded, host genotype-specific enhancement of bacterial fitness. J Bacteriol 1994;176:3140-7.
61. Abrams JS. Tamoxifen: five versus ten years—is the end in sight? J Natl Cancer Inst 2001;93:662-4.
62. Rudolph RE, Vaughan TL, Kristal AR, et al. Serum selenium levels in relation to markers of neoplastic progression among persons with Barrett's esophagus. J Natl Cancer Inst 2003;95:750-7.
63. Vaughan TL, Kristal AR, Blount PL, et al. NSAID use, BMI, and anthropometry in relation to genetic and cell cycle abnormalities in Barrett's esophagus. Cancer Epidemiol Biomarkers & Prev 2002;11: 745-52.
64. Corley DA, Kerlikowske K, Verma R, Buffler P. Protective association of aspirin/NSAIDs and esophageal cancer: a systematic review and meta-analysis. Gastroenterology 2003;124:47-56.
65. Ciagnard A, Martin DS, Michel MF, Martin F. Interaction between two cellular subpopulations of a rat colonic carcinoma when inoculated to the syngeneic host. Int J Cancer 1985;36:273-9.
66. Miller BE, Miller FR, Heppner GH. Therapeutic perturbation of the tumor ecosystem in reconstructed heterogeneous mouse mammary tumors. Cancer Res 1989;49:3747-53.
67. Moffett BF, Baban D, Bao L, Tarin D. Fate of clonal lineages during neoplasia and metastasis studied with an incorporated genetic marker. Cancer Res 1992;52:1737-43.
68. Akakura K, Bruchovsky N, Goldenberg SL, Rennie PS, Buckley AR, Sullivan LD. Effects of intermittent androgen suppression on androgen-dependent tumors. Cancer 1993;71:2782-90.
69. Sato N, Gleave ME, Bruchovsky N, et al. Intermittent androgen suppression delays progression to androgen-independent regulation of prostate-specific antigen gene in the LNCaP prostate tumor model. J Steroid Biochem Mol Biol 1996;58:139-46.
70. Buhler KR, Santucci RA, Royai RA, et al. Intermittent androgen suppression in the LuCaP 23.12 prostate cancer xenograft model. Prostate 2000;43:63-70.
71. Caldiroli M, Cova V, Lovisolo JA, Reali L, Bono AV. Antiandrogen withdrawal in the treatment of hormone-relapsed prostate cancer: single institutional experience. Eur Urol 2001;39 Suppl 2:6-10.
72. Scher HI, Zhang ZF, Nanus D, Kelly WK. Hormone and antihormone withdrawal: implications for the management of androgen-independent prostate cancer. Urology 1996;47:61-9.