Does folic-acid supplementation prevent or promote colorectal cancer? Results from model-based predictions

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Abstract

Folate is essential for nucleotide synthesis, DNA-replication and methyl-group supply. Low-folate status has been associated with increased risks of several cancer types, suggesting a chemopreventive role of folate. However, recent findings on giving folic acid (FA) to patients with a history of colorectal polyps raise concerns about the efficacy and safety of folate supplementation and the long-term health effects of folate fortification. Results suggest that undetected precursor lesions may progress under FA supplementation, consistent with folate's role in nucleotide synthesis and cell proliferation.

To better understand the possible trade-offs between FA's protective effects due to decreased mutation rates and possibly concomitant detrimental effects due to increased cell proliferation, we used a biologically-based mathematical model of colorectal carcinogenesis. We predict changes in cancer risk based on timing of treatment start and the potential impact of FA on cell proliferation and mutation rates. Conclusion: changes in colorectal cancer risk in response to FA supplementation are likely a complex function of treatment start, duration, and impact on cell proliferation and mutations rates. Predicted colorectal cancer incidence rates under supplementation are mostly higher than rates without FA supplementation unless supplementation is initiated early in life (before age 20). To the extent to which this model predicts reality, it indicates that the effect on cancer risk when starting FA supplementation late in life is small, yet mostly detrimental. Experimental studies are needed to provide direct evidence for this dual role of folate in colorectal cancer and to validate and improve the model predictions.

Introduction

The B-vitamin folate is essential for the synthesis of nucleotides as well as for the provision of methyl groups for the maintenance of DNA methylation in dividing cells (1). Low intakes of folate have been associated with increased risks of cancers of the colon, pancreas, esophagus, and possibly breast (2-4). The biologic mechanisms ascribed to these associations include higher mutation rates and reduced stability of DNA methylation patterns with a low folate status (1). However, the protective role of folate in carcinogenesis has recently been questioned and may be more complex and dependent on dose and timing of folate administration during the carcinogenic process (2, 3, 5). Animal experiments show that folate supplementation prior to the establishment of early neoplastic lesions reduces carcinogenesis, while administration after pre-cancerous lesions are present appears to increase tumor growth (2). Similarly, epidemiological evidence suggests that excessive levels of folate are not beneficial and may actually enhance cancer risk (3, 6). The recent polyp-prevention trial by Cole et al. is a case in point (7). This trial involved the administration of 1mg/day folic acid (the synthetic form of folate with greater bioavailability) to patients with a history of colorectal polyps. It provides first human evidence for a potentially detrimental effect of high dose folate in humans: not only was folic acid not chemopreventive, but an increased risk of advanced and multiple adenoma was observed in the intervention arm at 6-8 years of follow-up (advanced adenoma RR= 1.67; 95% CI: 1.00-2.80; $\geq$ 3 adenomas RR=2.32; 95% CI: 1.23-4.35). These results suggest that undetected precursor lesions were more likely to progress with folic acid administration (8).

The bimodal role of folate in carcinogenesis is thought to be attributable to its role in nucleotide synthesis. Rapidly proliferating tissues, including tumors, have a greater requirement for folate. Cancers often overexpress folate receptors and genes in nucleotide synthesis to meet their increased need of sustained DNA synthesis and proliferation (9-11). Antifolate drugs have been successfully used in cancer chemotherapy and in the 1940s folate has been described as inducing an “acceleration phenomenon” if provided to children with leukemia, suggestive of increased progression (12).

The question arises whether folate intakes in the population may approach levels that could cause

harm. Approximately 30-40% of adults in the United States use nutritional supplements that contain folic acid, with a standard multivitamin containing 400 μg (13). Ironically, the group with the highest supplement use is comprised of older individuals, who are also more likely to have precancerous lesions. For example, colorectal polyps are thought to exist in about 30% of adults 60 years and older, but in many fewer younger individuals (14, 15). In addition to supplement use, a number of functional foods are fortified with folic acid, including nutrition bars and drinks (often at 400 μg/serving), as well as fortified breakfast cereals. Finally, the United States initiated folic acid fortification of grain products, allowable in 1996 and mandatory as of Jan 1, 1998, to reduce the incidence of neural tube defects. This public health measure has been highly effective in reducing neural-tube defects (16), yet has also increased the folic acid intake universally in the US in the population (beyond the target group of women of childbearing age) by about 100-200 μg/day (17). Biomarkers of folate intake suggest that a significant fraction of the population has now folate levels that have been previously considered "supraphysiological", presumably due to a combination of fortification and supplement use (17).

The increase in folate status in the population and its potentially dual role in colorectal carcinogenesis has raised the question whether folic acid fortification will prevent or promote colorectal cancer (18). Considering the multiple, opposing effects on cancer risk, the answer is not straightforward. We approached this complex question by using a mathematical model for colorectal carcinogenesis. The model uses 4 stages to describe the progression to a colorectal polyp that grows and transforms into a carcinoma. Results from this modeling strategy have previously been shown to match Surveillance, Epidemiology and End Results (SEER) incidence data of colorectal cancer (19). We utilize this model to investigate the "net impact" of effects of folate supplementation on mutation rates and cellular proliferation on colorectal cancer rates in the population.

Model and methods

To explore colon cancer risk in response to changes in folate status (e.g. folate supplementation) we utilize a mathematical model that mirrors the multistage nature of colorectal cancer including salient features of its pathogenesis. Specifically, we use the multistage clonal expansion model

developed by Luebeck and Moolgavkar (19), a model which has been shown to be consistent with the observed incidence of colorectal cancer in the general population. The model stipulates three distinct phases in the process of carcinogenesis. In the first phase, that of initiation, a susceptible stem cell acquires one or more mutations resulting in an initiated cell, which has partially escaped growth control. The second phase, that of promotion, is the clonal expansion of initiated cells. Promotion is an extremely efficient way of bringing about carcinogenesis because clonal expansion results in increased populations of cells that have already acquired some of the genetic alterations on the pathway to malignancy. In the last phase, that of malignant conversion, an initiated cell acquires another genetic change, one required to convert it into a malignant cell. There is considerable evidence that most human malignancies go through these three phases and that environmental agents, such as radiation and tobacco smoke, influence carcinogenesis via their effects on one or more phases of this process (20, 21).

Fig.1 provides a schematic view of the colon carcinogenesis model. The model assumes that the formation of an adenoma requires biallelic inactivation of a tumor suppressor (or 'gatekeeper') gene, such as the APC gene. It also assumes sustained (asymmetric) stem cell divisions of a mutant stem cell progenitor represented in the model by a high-frequency event. These divisions represent the sustained generation of mutant progeny (via transient amplification) by a mutant stem cell located at the bottom of a colonic crypt. Therefore, according to this model, a mutant stem cell will only undergo clonal expansion (or promotion) once it leaves the protective environment of the stem cell compartment and moves toward the top of the crypt – and the rate of clonal expansion is determined by the net cell proliferation parameter α-β (Fig.1) (22, 23). Finally, a carcinoma develops from an adenoma in a single rate-limiting event (the 'adenoma to carcinoma' transition), which in this context is also referred to as malignant transformation.

For the purpose of this study, we have extended the mathematical formulation of this model to accommodate time-dependent model parameters reflecting the changes that might occur in an individual's folate status. For simplicity, we assume that a change in folate intake from one level to another immediately effects constant changes in the model parameters ignoring possible delays in the cellular and enzymatic response to folate. Mathematical details of the model and a derivation of the age-specific hazard function and tumor probabilities can be found in the

supporting information of Luebeck and Moolgavkar (19, 24). The extension of the hazard function from constant parameters to piece-wise constant parameters is provided in the Appendix. R-code for computing the hazard function and tumor probabilities can be obtained from the authors upon request.

First, we use the mathematical model to explore the relative effects of folate-induced changes in mutation rates and cell proliferation rates (of stem cells in adenomas) on colon cancer risk (Figure 1). Here we are interested in quantifying the trade-offs between potentially opposing effects of folate (or folate supplementation) on the carcinogenic process: reduction of mutation rates (e.g., by reduced uracil misincorporation into DNA) versus stimulation of cell proliferation of intermediate (adenomatous) cell populations at risk for malignant transformation. Specifically, we assume that increases in the cell proliferation rate α (see Fig. 1) translate into proportionate increases in the net proliferation (promotion) parameter α-β. This assumption is equivalent to assuming that both cell proliferation and cell death (apoptosis) are equally affected by folate. However, it is possible that folate reduces the rate of cell death yielding even a stronger effect on tumor promotion. Thus, assuming that the percent increase in cell proliferation equals the percent increase in net cell proliferation (or promotion), we make a conservative assumption concerning a possible tumor-promoting effect of folate in carcinogenesis.

Second, we explore the predicted time course of colon cancer risk and its dependency on the age at which folate supplementation begins. Finally, since the magnitude of both folate's effects on mutation rates and cell kinetics are uncertain and likely depend on folate dose and genetic make-up of the individual, we explore the sensitivity of the cancer risk to independent variations in both mutation rates and net cell proliferation (promotion) rates. For simplicity, we assume that all three mutation rates ($\mu 0$, $\mu 1$, and $\mu 2$) in the model are equally affected by folate, an assumption that seems plausible for the first two events representing mutations at the APC locus. However, considering that the adenoma-carcinoma transition (rate $\mu 2$) is a more complex process that involves malignant transformation and the dynamics of tumor progression, there is more uncertainty regarding the response of $\mu 2$ to folate.

Results

Figure 2 (panel A) shows the impact of reduced mutation rates, alone or in combination with increased cell proliferation rates, on the relative risk of colon cancer as a function of age when supplementation commences early in life (age 2). For completeness we also show the risk when cell proliferation is increased without concomitant increases in mutation rates. Panel B shows the same scenarios as panel A, but for the predicted excess number of colon cancers per 100,000 individuals at risk. Assuming that folate has no effect on cell proliferation, a 20% reduction in mutation rates translates into an approximately 40% reduction in relative risk about 10-20 years after start of treatment. In contrast, a concomitant 20% increase in cell proliferation is seen to interfere with the protective effect from reduced mutation rates in a complex age-dependent manner. In this scenario, with the exception of an early (transient) reduction of relative risk before age 30, a significant/meaningful reduction in relative risk does not occur until much later in life (solid line). The potentially rapid drop in relative risk soon after start of folate supplementation (age 2 in Fig.2) can be attributed to the reduction in the adenoma-carcinoma transition rate, the rate-limiting event representing malignant transformation and (in the model here) immediate clinical detection of cancer. In spite of temporarily increased relative risks due to possible opposing effects of folate on mutation rates and rates of cell proliferation in colorectal adenoma (as shown in Panel A), the impact on the cumulative cancer risk (as shown in panel B) is much less pronounced but may still lead to several thousand excess cancer cases (per 100,000 individuals at risk) for individuals age 60 to 70. This example demonstrates that the effect of genomic protection that folate (or folic acid) is thought to exert on normal and cancerous cells could be canceled (at least partially) by an increase in cell proliferation of similar magnitude.

Timing of folate supplementation.

Figure 3 shows the predicted number of excess cases of colon cancer (in the absence of competing causes) as a function of current age for 4 different ages at which supplementation begins (ages 2, 20, 40 and 60) assuming a constant folate dose. All scenarios assume that folate supplementation reduces the APC mutation rates (i.e. the rates of the first two rate-limiting events) and the malignant transformation rate by 20%, but increases the cell proliferation rate by

20% compared to (untreated) controls. It can be seen that the cancer risk is mostly higher than the background risk (without supplementation) unless folate supplementation begins early in life. On the other hand, it is intuitively clear that, when folate is given late in life, the effect on cumulative risk will be small as the majority of cancer cases occur before treatment begins. Qualitatively similar curves are obtained for a 10%/10% scenario (results not shown). The main conclusion drawn here is that the risk of colon cancer would mostly be higher than the background risk (without supplementation) unless folate supplementation is begun early in life (well before age 20). When folate supplementation is started late in life, its effect on the cumulative cancer risk appears to be small and mostly detrimental.

Sensitivity of colorectal cancer risk to (hypothetical) variations of mutation and cell proliferation rates.

The magnitude of folate-associated anti-carcinogenic and carcinogenic effects is highly uncertain. They likely depend on folate dose (e.g., fortification versus supplementation) and genetic make-up of the individual (25, 26). To address this uncertainty on the level of the cell (and within the framework of multistage carcinogenesis), we explore the sensitivity of the (life-time) cancer risk to variations in both mutation rates and cell proliferation rates (Fig.4). To keep the discussion simple, we assume (see Models and Methods) that the percent increase in cell proliferation equals the percent increase in net cell proliferation (or tumor promotion) which is the main determinant for tumor growth. The predicted cancer risk, again in terms of the expected number of excess cases by age 70, responds almost linearly to moderate reductions (between 0 and 40%) in mutation rates and increases in proliferation in the same range, unless supplementation is started early in life. When started early in life (Fig.4 top panel), the risk surface responds exponentially to changes in mutation rates. The enhanced sensitivity of the cancer risk with very early folate exposure reflects the more prolonged folate intake and higher cumulative folate dose compared with later starting points. Comparison of the 3 scenarios shown in Fig.4, especially in regard to the predicted (life-time) reductions in the number of colon cancer cases in response to decreases in mutation rates, show that an efficient reduction in cancer risk – in the presence of significant (putative folate-induced) increases in the growth rate of the adenomatous polyps – likely occurs only when the supplementation starts very early in life.

Of particular interest is the nullcline (yellow curve in Fig.4), the curve for which reductions in mutation rates and concomitant increases in tumor promotion cancel each other out yielding no change in cancer risk. In addition, we also highlight two specific 'trade-off' scenarios (labeled 'A' and 'B' on the surfaces shown in Fig.4) for which the percentage decrease in mutation rates equals the percentage increase in net cell proliferation (or promotion). Specifically, the point 'A' represents a 20/20 modulation, and the point 'B' a 30/30 modulation. Inspection of the respective distances of points 'A' and 'B' from the nullclines (for the 3 scenarios shown in Fig.4) reinforces the notion that, unless folate supplementation is begun very early in life, the risk of colon cancer may well be increased if folate promotes premalignant lesions on the pathway to cancer. Furthermore, the point 'B', which represents a higher sensitivity, is pushed toward higher risks (away from the nullcline) compared to point 'A' when the onset of folate supplementation is delayed for 2 or more decades.

Table 1 provides direct estimates for this sensitivity analysis, giving predicted rates of colorectal cancer in the population depending on multiple scenarios. It illustrates how the expected number of colorectal cancer cases changes depending on proliferation rate, mutation rate, and starting age of folic acid supplementation use. For example, compared to the “baseline” of 3,319/100,000 cases per year, there are substantial reductions in the number of cases if the mutation rate is reduced by 20%, independent of age group. If concomitantly proliferation increases (scenario: +10% proliferation / -20% mutation rate), then only the youngest age group still benefits (-455 cases) whereas individuals who were 20 or 30 years old at the initiation of supplementation would show increased cancer rates. If the proliferation increase is more substantial (scenario: +20% proliferation / -20% mutation rate) then increases in cancer rates are expected in all age groups (+419/100,000 for those who were 2 years old at supplementation begin, + 1053/100,000 for those who were 40-years old).

Discussion

Here we use a mathematical modeling strategy to investigate the “net effect” of folate administration on colorectal cancer incidence in the general population, varying the age at which

supplementation is started as well as the putative effects of folate on mutation rates and net cell proliferation. Naturally, this model can only provide predictions that need to be tested further in experimental and epidemiologic settings. However, in consideration of potential harmful effects of folate in subsets of the human population, many study designs may not be ethical or feasible. The specific examples proffered here are clearly hypothetical, but construed to allow an assessment of uncertainty and sensitivity of cancer risk in response to specific folate effects. Our examples address two questions, (1) what are the relative biological effects of folate-induced reductions in mutation rates versus increases in cell proliferation, either in isolation or concomitantly, and (2), how does the cancer risk associated with folate supplementation vary with age at the initiation of supplementation?

Results from our modeling suggest that the age of initiation of folate supplementation is critical. Unless folate supplementation begins very early in life, there appears little benefit of folate supplementation and possible harm, if one assumes equal effect sizes (e.g. 20% mutation reduction and a 20% proliferation increase). How quickly any potential (population-level) benefit may be lost, when folate supplementation does not start very early in life, can be gleaned from Fig.3. When the start occurs between ages 20 and 40, the excess number of cancer cases reaches about 2000/100000 at age 80, and is still at about 1000/100000 excess cases at age 80 when folate supplementation starts at age 60.

The sensitivity analyses that explore various effect sizes for folate on mutation reduction and on cell proliferation illustrate the potency of a promotional effect of folate on proliferation. Even when folate supplementation is started in young adulthood (around age 20) our model calculations suggest that a 10-15% promotional effect of folate is sufficient to eliminate any protective effect associated with a 40% reduction of all three critical mutation rates in the model (i.e. the rates of both APC mutations and the rate-limiting event defining the adenoma-carcinoma transition). These results should raise concerns, even if there is significant uncertainty in our knowledge of the tumor- or growth-promoting effects of folate. Considering that a very modest increase in cellular proliferation with folate may have significant adverse effects on colorectal cancer rates in the population, we really need better quantitative data to establish the extent of such effects. It is conceivable that such effects only occur at an overall excess dose, or that they

314 are more pronounced with folic acid rather than natural folates. It is unlikely that such data can
315 be generated from human studies, but may be generated from animal studies under controlled
316 environments and exposures (2).
317
318 Our model suggests that, in individuals who are older when supplementation is initiated, an
319 increase in colorectal cancer rates is expected to be seen after a period of about 10 years (see Fig.
320 3). These data appear consistent with recent data from the SEER cancer registries in the United
321 States and Canada presented by Mason et al. (27). In this ecological study, shortly after the
322 initiation of fortification, colorectal cancer rates increased in both countries resulting in an
323 annual excess of ~4 to 6 additional CRC cases per 100,000 individuals at risk. This abrupt
324 increase in cancer risk may simply reflect the accelerated development and growth of a nascent
325 malignancy into a clinically detectable or symptomatic cancer. Although our model does not
326 explicitly describe this process (which may be considered part of the adenoma-carcinoma
327 transition described by the last step in our model), it allows for accelerated growth of benign
328 adenomas on the pathway to cancer (promotion). Our model predicts an excess CRC risk that
329 increases with time about 10 years after supplementation is initiated (Fig. 3). Note, however,
330 Mason et al. only studied a period of 7 years (1996-2002) of folate fortification (27) and to
331 answer the question whether or not the observed increase in CRC risk is transient, constant, or
332 will eventually increase as predicted by our model will require more follow up data.
333
334 There are several uncertainties to our modeling. Although folate deficiency induces decreases in
335 cellular proliferation, there is no direct support for increased cell replication *in vivo* in the
336 presence of excess folate. However, in vitro studies show that cellular growth arrest induced by
337 folate deficiency can be reverted (after some delay) under acute folate repletion (28). Moreover,
338 possible changes in the rate of cell differentiation and apoptosis, which have been only poorly
339 studied in this context, may also play a decisive role in promoting tumor growth. Note that
340 accelerated tumor growth may result either from increases in the rate of cell replication, or from
341 decreases in the rate of apoptosis, or delays in cell differentiation. A recent study of folate
342 supplementation on mucosal cell proliferation in high risk patients for colon cancer is of interest
343 regarding the latter possibility (29). In this study it was found that folate supplementation mainly
344 decreased cell proliferation in the luminal part of the colorectal crypts, reflecting defective cell

differentiation control and delayed onset of normal cell differentiation and, ultimately, apoptosis. Such delays may effectively increase tumor size.

The effects of folate on mutation rates are also not well quantified. Folate deficiency increases the misincorporation of uracil into DNA damage, which can cause DNA strand breaks during repair. However, because methylated CpG sites are mutational hotspots for C>T transitions, moderate folate deficiency, which reduces, albeit slightly, genomic DNA methylation, may also protect against this type of mutation, as has been suggested for the MTHFR 677 TT genotype (30). Thus, the relationship between folate and mutation rates still needs to be better defined.

We are well aware of the hypothetical nature of this investigation. For one, the model used here, although broadly consistent with important biological processes involved in colorectal cancer and numerically consistent with the observed incidence of colorectal cancer in SEER (19), is fraught with considerable uncertainty. For example, not all biological parameters of the model can be estimated from incidence data alone making it necessary to fix one or the other model parameter [see Appendix]. Furthermore, the number of clonogenic (transformable) stem cells in colon remains elusive (with estimates from a few stem cells to hundreds of stem cells per colonic crypt (31-33). More concerning, however, is the uncertainty due to multiple 'modes of action' of folate on cancer initiation, cell proliferation and tumor progression. Much experimental and clinical work remains to be done to define and to quantify the beneficial and detrimental effects of folate on cancer risk, in particular how these effects are mediated on the cellular level. Our calculations suggest that a focus be given to a careful study of possible proliferative effects of folate on precursor lesions such as the adenomatous polyps in colon. We propose that this question first be studied in a rodent model where the number and sizes of premalignant and malignant lesions can be readily measured and cell proliferation kinetics ascertained with immuno-histochemical techniques. Mouse endoscopy techniques provide now tools to evaluate the impact of folate on existing polyps. As noted above, these cancer precursors are very common (14), yet often go undetected due to a lack of appropriate colorectal cancer screening. Only about 30% of US adults over age 50 have had a screening colonoscopy within the previous 5 years (34). As noted previously, the Aspirin/Folate Prevention Trial (7) only enrolled individuals with prior resected adenoma and full colonoscopies. Thus, it does not provide

answers regarding the effects of folate on existing polyps, which may potentially be much stronger than those on newly arising (or undetected) polyps, that were observed within the trial during follow-up colonoscopy (8).

In the United States, some have suggested that a further increase in the amount of folic acid in fortified foods is warranted. Similarly, several European countries are considering the introduction of folic acid fortification for the prevention of neural tube defects. Our results add another important piece to inform this public health policy debate. A key message is that – if excessive folate has tumor-promoting effects, then those are likely to outweigh any beneficial effects of folic acid supplementation on mutation rates with cancer rates predicted to rise in all but those treated at a very young age. These results suggest caution when considering the implementation of fortification until we have better data on the effects of folate on cell kinetics, tumor promotion, and their quantitative effects by dose and type of folate.

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Figure 1. CRC model: biallelic inactivation of the APC gene is assumed to occur in colonic stem cells in two rate-limiting steps with rates μ0 (for the first allele) and μ1 (for the second allele). After the first step the crypt may contain a mixture of APC-wild-type and APC+/- cells (nuclei represented by filled and open circles, respectively). Transient amplification with production rate ρ populates the proliferative zone above the stem cell compartment with APC-/- progeny (grey cells with square-shaped nuclei). Unresponsive to changes in Wnt-signaling, this progeny remains in a proliferative state as it enters the differentiation zone near the top of the crypt leading to rapid accumulation of APC-/- cells. Subsequent clonal expansion of APC-/- cells, which divide with rate α and die or differentiate with rate β, describes the growth of an adenoma. The final event in the model represents the adenoma-carcinoma transition, which occurs with rate μ2. A reduction in the mutation rates with folate supplementation is modeled by a % decrease of μ0, μ1 and μ2. Effects on replication rates by folate supplementation are modeled with a % increase in the replication rate α. Folate supplementation is assumed to decrease the mutation rates μ0, μ1 and μ2, but increases the cell division rate α and the transient amplification rate ρ. The ratio β/α is assumed constant, thus only the net cell proliferation rate α – β increases with folate supplementation.

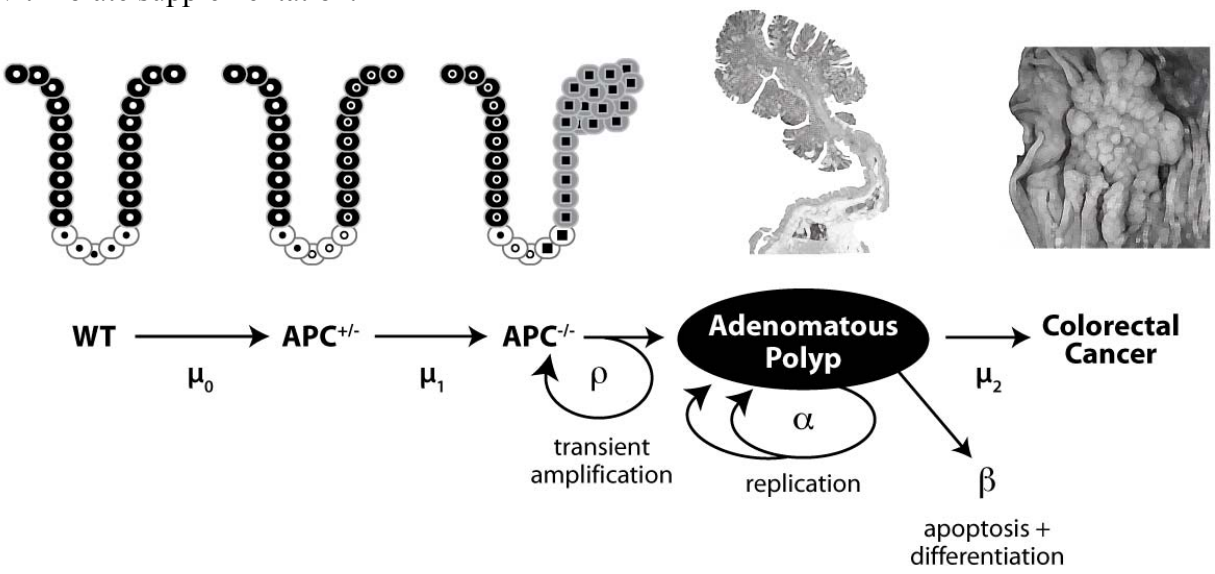

Figure 2. A) Relative risk of colon cancer as a function of current age for when supplementation begins at age 2 with different combinations of reduced mutation rates and increased cell proliferation rates. **B)** Predicted excess number of colon cancers per 100,000 individuals at risk as a function of current age for when supplementation begins at age 2 with different combinations of reduced mutation rates and increased cell proliferation rates.

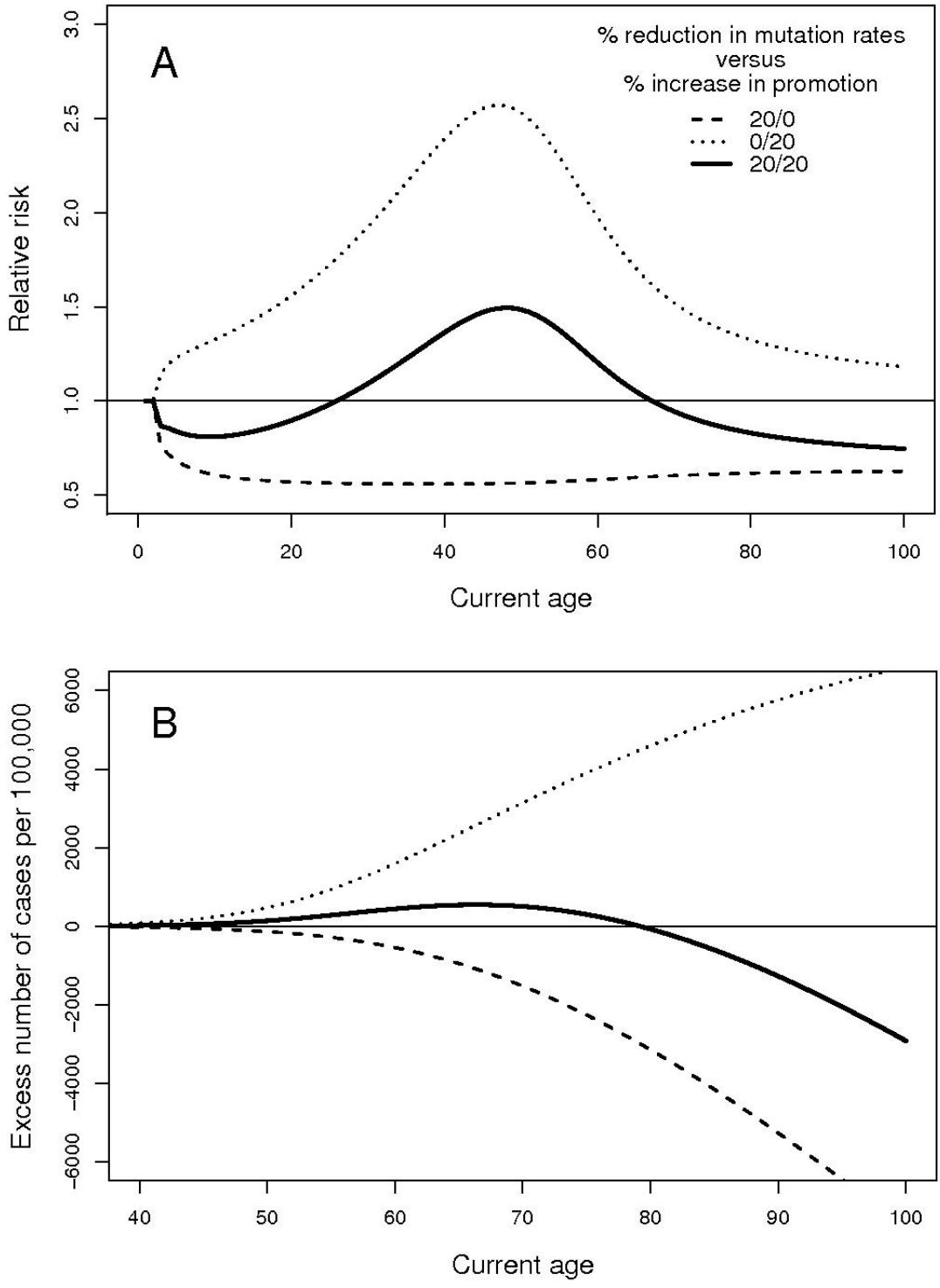

Figure 3. Predicted number of excess cases of colon cancer as a function of current age for different ages at which supplementation begins.

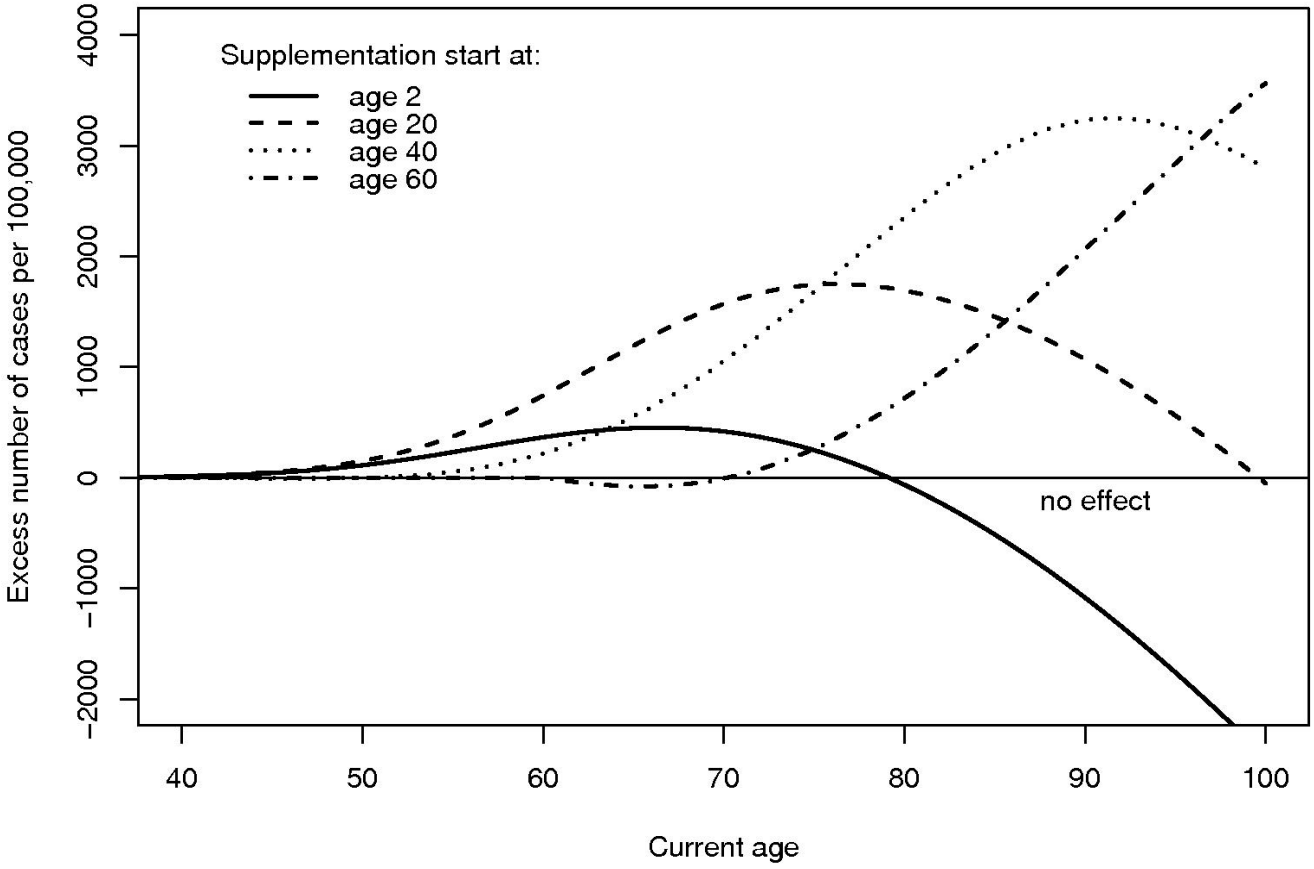

Figure 4. Predicted number of excess cases of colon cancer as a function of both mutation rates and cell proliferation rates for different ages at which supplementation begins.

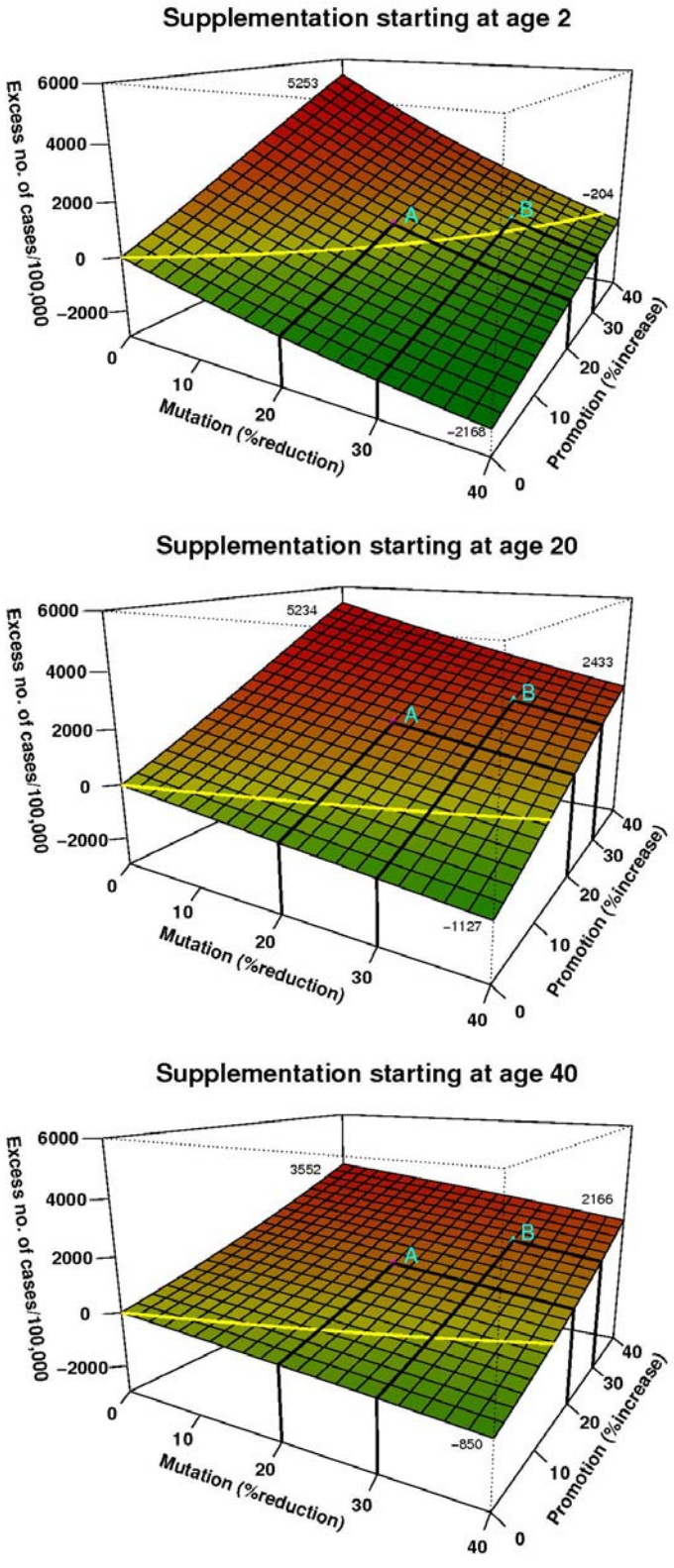

Table 1. Expected number of colorectal cancer cases depending on proliferation rate, mutation rate, and starting age of folic acid supplementation use.

Proliferation Increase (%)	**Mutation Reduction (%)**	**Folic acid supplementation starting at age**		
		2	20	40
		Expected number of colorectal cancer cases per 100,000 individuals at risk		
0	0	3319	3319	3319
		Change in colorectal cancer cases		
0	10	-687	-298	-193
0	20	-1274	-583	-397
10	0	+1286	+1223	+732
10	10	+353	+826	+511
10	20	-455	+449	+275
20	0	+2631	+2565	+1571
20	10	+1451	+2051	+1320
20	20	+419	+1570	+1053

Appendix

For the class of models where initiation of a premalignant lesion (such as an adenomatous polyp in the colon) requires a number of rate-limiting events to occur in specific order, as for the model presented in Fig.1, the tumor survival function is readily derived from the probability generating function of a filtered Poisson process (35). Specifically, let $S_k(s,t)$ represent the survival function of a filtered (non-homogeneous) Poisson process that starts off at time s for a model with k steps. Assume that cells of type 0 (normal cells) emit cells of type 1 with rate μ_0, cells of type 1 emit cells of type 2 with rate μ_1, ..., until cells of type k–2 emit cells of type k–1, which may undergo clonal expansion before giving rise to malignant progeny (type k). For this model class, it is easy to show that the survival function can be computed recursively:

$$S_j(s_{k-j},t)=\begin{cases}\exp\left[-\int_{s_{k-j}}^{t} ds_{k-j+1}\mu_{k-j}(1+X\delta_{j,k})\left(1-S_{j-1}(s_{k-j+1},t)\right)\right] & \text{if } 2<j\le k\\ \left[(q-p)/\left(qe^{-p(t-s_{k-j})}-pe^{-q(t-s_{k-j})}\right)\right]^{\mu_{k-j}/\alpha} & \text{if } j=2\end{cases}$$

Here, δ_{ik} is the Kronecker symbol, X is the number of normal stem cells in the tissue, and the parameters p and q are related to the biological parameters α (the cell division rate), β (the cell death rate), and the malignant transformation rate μ_{k-1} via

$$p=\frac{1}{2}\left(\left(-\alpha+\beta+\mu_{k-1}\right)-\sqrt{\left(\alpha+\beta+\mu_{k-1}\right)^2-4\alpha\beta}\right)$$
$$q=\frac{1}{2}\left(\left(-\alpha+\beta+\mu_{k-1}\right)+\sqrt{\left(\alpha+\beta+\mu_{k-1}\right)^2-4\alpha\beta}\right).$$

Note, $g\equiv-(p+q)=(\alpha-\beta-\mu_{k-1})$, is approximately equal to the net cell proliferation rate for the clonal stage (stage k–1) of the multistage process. The parameter q is approximately equal to $\mu_{k-1}/(1-\beta/\alpha)$, which may be viewed as an upper bound for the malignant transformation rate. See Heidenreich et al. (1997) for more details.

The age-specific hazard function $h_k(t)$, required for the analysis of cancer incidence in populations, can now be derived from the survival function by computing

$$h_k(s_0,t)=\frac{\partial}{\partial t}\ln S_k(s_0,t),$$

which readily yields

$$h_k(s_0,t) = \int_{s_0}^{t} ds_1 \mu_0 X \; S_{k-1}(s_1,t)) h_{k-1}(s_1,t).$$

Again, the first time argument of (s, t) makes explicit the time origin of the stochastic process in question. Thus, for a 4-stage model, we have (now dropping the argument $s_0 = 0$)

$$h_4(t) = \int_0^t ds_1 \mu_0 X \; S_3(s_1,t) \int_{s_1}^{t} ds_2 \mu_1 S_2(s_2,t) h_2(s_2,t),$$

where h_2 and S_2 are the hazard function and survival function of the two-stage clonal expansion (TSCE) model, respectively. See Heidenreich, et al. (1997) for explicit formulas for h_2 and S_2 for the case of constant or piecewise constant parameters. For constant parameters, setting $\mu_0=\mu_1=\nu$ (i.e. equality of the two APC mutation rates), the hazard function for our colon cancer model (see Fig.1) simplifies to

$$h_4(t) = \nu X \left(1 - \exp\left\{\int_0^t du\, \nu\left(S_2(u,t) - 1\right)\right\}\right).$$

When the parameters are time-dependent, as may be the case with drug treatment or exposures to chemo-preventions such as NSAIDs or folate, then we use the more general formula for $h_4(t)$ and evaluate the time integrals numerically by integrating from time t to 0 for efficiency.

Note, X, the number of normal stem cells, always appears in combination with $\mu_0(= \nu)$, and therefore cannot be determined without further assumptions or constraints on the parameters. For more details on parameter identifiability see e.g. Hanin and Yakovlev (1996) and Heidenreich et al. (1997).

We have fitted the 4-stage model to the incidence of colorectal cancers reported in the SEER registry (1973-2000) following the approach first described in Luebeck and Moolgavkar (2002). Our parameter estimates differ only slightly from those of the earlier analysis. We now include 4 additional calendar years (1997-2000). Specifically, for the calculations presented here, we use the estimates $\nu=1.4910^{-6}$, $\rho(=\mu_2)/\alpha= 1.84$, $p \approx (\alpha-\beta)=0.155$, and $q =1.205\ 10^{-5}$. As in the earlier analysis, we assume a constant number of stem cells in the colon, $X = 10^8$, which is also similar to the value provided in Potten et al. (2002).