Nutrition

Cancer Prevention and Treatment by Wholistic Nutrition

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Cancer is traditionally considered a genetic disease. It starts with a gene mutation, often caused by environmental carcinogens that are enzymatically activated to metabolites that covalently bind to DNA. If these now-damaged carcinogen-DNA adducts are not repaired before the cell replicates, they result in a mutation, which is inherited by daughter cells and their subsequent progeny. Still more mutations are added that are thought to advance cellular independence, metastasis, and drug resistance, among other characteristics typically observed for advanced cancer. The stages of initiation, promotion and progression of cancer by mutations infer irreversibility because back mutations are exceedingly rare. Thus, treatment protocols typically are designed to remove or kill cancer cells by surgery, chemotherapy, immunotherapy and/or radiotherapy. However, empirical evidence has existed to show a fundamentally different treatment option. For example, the promotion of cancer growth and development in laboratory animals initiated by a powerful mutagen/carcinogen can be repetitively turned on and off by non-mutagenic mechanisms, even completely, by modifying the consumption of protein at relevant levels of intake. Similar but less substantiated evidence also exists for other nutrients and other cancer types. This suggests that ultimate cancer development is primarily a nutrition-responsive disease rather than a genetic disease, with the understanding that nutrition is a comprehensive, wholistic biological effect that reflects the natural contents of nutrients and related substances in whole, intact food. This perspective sharply contrasts with the contemporary inference that nutrition is the summation of individual nutrients acting independently. The spelling of 'holism' with the 'w' is meant to emphasize the empirical basis for this function. The proposition that wholistic nutrition controls and even reverses disease development suggests that cancer may be treated by nutritional intervention.

cancer treatment | cancer mutations |
cancer etiology | cancer prevention

Introduction

A new perspective is needed on the failed War on Cancer begun 46 years ago by President Nixon because there is little or no convincing evidence that this project has specifically decreased the all-important rates of cancer. There certainly has been progress, however, in understanding this exceptionally complex disease. Newer methods for research and possible treatment protocols have been developed. For example, much has been learned about the fascinating but especially complex genetic basis for cancer and clever proposals have been made to re-engineer components of the immune system to treat cancer(*1*). But these advances have not yet changed overall cancer rates or have identified a 'cure' for cancer.

Cytotoxic chemotherapy, radiotherapy and surgery are still the traditional treatments of cancer, hopefully leading to the selective killing of cancer cells while minimizing damage to the neighboring normal cells. Except for the promises offered by novel versions of immunotherapy and a better understanding of the underlying genetics, cancer is still on the public's mind as the same costly, dreaded disease that it has always been. Consider, for example, an ominous 2004 report showing that cytotoxic chemotherapy (for 22 types of cancer) increases 5-year survival only by 2.1%(*2*) while the cost of developing a new cancer drug approximates $2 billion. This cannot sustain public support, especially when a placebo effect might be as high or higher than the 2.1%.

Having been in the cancer research community for more than 60 years, I am well aware that progress in controlling this extraordinarily complex disease has been difficult. I contend that it will stay difficult, however, because of an inadequate understanding of its basic biochemistry as well as the basic biochemistry of nutrition, which is a major effector of cancer development. I suggest that this concept of complexity for both disciplines is seriously underestimated or, at best, is seriously oversimplified, thus the association of diet with cancer cannot be fully understood. For example, consider the landmark 1981 report on diet and cancer submitted to the Office of Technology Assessment of the U.S. Congress which concluded that 35% of total cancer was attributed to diet(*3*), with estimates by some authorities surveyed for that report being as high as 70%. The 35% estimate of diet-attributable cancer has been widely cited by many authorities and institutions ever since 1981, often as the dietary causation of one-third of all cancers. But this evidence mostly referred to associations of specific nutrients with specific cancers. Is it possible that that this is an over-simplification of this obviously complex diet-cancer relationship? Also, does the well-established belief that cancer is a genetic disease moot the nutritional contribution to cancer? The objective of this paper therefore is to encourage discussion of the fundamental relationship of nutrition with cancer, mainly centered on the unusual complexity of the underlying biology of each process.

Most conventional wisdom holds that research interest in the effect of diet on cancer began in earnest during the 1940s to 1960s (summarized by the National Academy of Sciences in 1982)(*4*). Laboratory animal studies were showing that experimental tumor formation, initiated by chemical carcinogens, was increased by consumption of nutrients like fat, animal protein and/or calories,(*4*) which generally represented the effects of diets rich in animal-based foods.(*4, 5*) The emphasis was on the cancer modifying (not initiating) effects of nutrients, which were mostly investigated as single agents in laboratory animal experiments.

Later, in the 1960s and 1970s, diet and cancer research turned toward human population studies that correlated cancer rates with foods, generally explained by their nutrient contents(*6-8*) The most cited correlations were those of dietary fat with breast(*7, 9-14*) and colon cancers(*15, 16*) and dietary fiber with large bowel cancer(*17, 18*) The consumption of nutrients of animal-based foods were associated with increased cancer risk while nutrients of plant-based food were associated with decreasing risk.

Conflict of Interest: No conflicts declared.
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However, instead of nutrients explaining the association of food with cancer, another school of thought suggested that certain non-nutrient (environmental) chemicals in food were more responsible, especially those that caused genetic mutations. This concern with environmental chemicals was highlighted with the so-called cranberry scare during the late 1950s when evidence suggested that a herbicide sprayed on cranberries, aminotriazole, caused thyroid cancer in laboratory animals, a highly publicized report that almost wiped out the cranberry season that year.(*19*) The cancer properties of environmental chemicals became highly publicized and politicized, resulting in a 1958 amendment to the Food and Drug Act, famously called the Delaney Clause, which demanded zero tolerance of chemical carcinogens in food (reviewed elsewhere(*20, 21*)). Also, at about this same time, the publication of the popular book "Silent Spring" by Rachel Carson generated widespread public attention on the possible health hazards of environmental chemicals for human health and, in the views of many, led to the founding in the early 1970s of the U.S. Environmental Protection Agency.(*22*)

Somewhat before this time (1950s), in a related development, the Watson and Crick model for DNA structure and function was elucidated, cementing the idea that all biological events arise from genes and, for cancer, probably from genes that were mutated. This convergence of events (mutation of cancer by environmental chemicals and associations of diet with cancer), therefore, required a technology to search for and test environmental chemicals (mostly in food) that might cause cancer in humans. Because no simple lab-based method was available for this purpose, an animal bioassay program(*23-25*) was developed, the results of which were used to estimate human cancer risk contributed by these environmental chemicals.(*25*)

This bioassay program gradually evolved over 2-3 decades, involving the participation of three government agencies (two US, one WHO). Experimentally, this bioassay program required 1) the testing of candidate chemicals in both sexes of two species of animals (mostly rats and mice but sometimes dogs and/or monkeys in earlier years), 2) four experimental groups (dietary control group, a group fed 100 times the amount of suspect chemical anticipated for human food, a group fed the maximum tolerated dose of this chemical, and an intermediate dose group), 3) two-year lifetime studies, and 4) a histological search for tumors at the end of the study.(*26*) Although the cost of testing one candidate chemical in 1961 was $10,000 to $15,000, it eventually rose to $2-4 million in 2009, based on the testing for carcinogenicity in two species.(*26*)

As time passed, this bioassay program also became more comprehensive in order to include other toxicological responses. Certain pharmaceutical products, like oral contraceptives, require longer test periods up to ten years and the use of species (dogs, monkeys) that were deemed more appropriate for human comparison. In recent years, efforts have been made to refine dose selection, number of dose groups, control group characteristics, choice of animal species/strain and duration of study, among other experimental parameters.(*26, 27*)

Estimates of human risk from the results of this bioassay program requires both high dose to low dose interpolation and species to species extrapolation, which have been highly debatable exercises.(*28, 29*) For much of the earlier history of this program, it also was assumed that candidate chemicals for testing were those that cause mutations to initiate cancer. This program continues until the present day(*27*) although more recently, chemicals capable of promoting cancer without being mutagenic also may be called carcinogens.(*30, 31*) This program is now more than a half-century old but it is still beset with concerns about its purpose and its relevance, as was first questioned more than three decades ago.(*20, 32, 33*) A decade later, in 1995, it was referred to as "archaic cancer research" but it still survives, having created a profound belief in the idea that it is our exposure to environmental chemicals that chiefly cause human cancer}.(*33*)

Explaining how food associates with cancer during the past half-century, therefore, fundamentally depended on two hypotheses, one focused on environmental chemicals that are mutagenic and carcinogenic, the other on nutrients that are not mutagenic. There is no doubt that most public interest in the food and cancer connection was and still is focused on carcinogens that cause mutations. Aside for the testing of single chemicals in the animal bioassay program, this led many years ago to the development of non-animal, short-term, lab-based assays, the most popular being the Ames assay,(*34, 35*). It tested the in vitro ability of suspect chemicals to cause mutations in bacterial and other cell cultures, perhaps as a surrogate or as a replacement for the animal bioassay.

Because nutrients are not mutagenic and are not investigated by this bioassay program, other mechanisms for their cancer enhancing effects were sought during the 1970s and 1980s. A private research organization, the American Health Foundation, became particularly active in seeking mechanistic explanations, investigating, for example, how circulating estrogens(*36, 37*) might explain dietary fat and fiber effects on breast cancer,(*38, 39*) and how bile acid activities(*40*) might explain large bowel cancer. Findings from other researchers concerned the effect of calorie consumption and energy metabolism on various cancers(*41, 42*)and the antioxidant activity of vitamin A,(*43*) especially β-carotene,(*44*) on lung cancer. In laboratory rodent studies, animal-based protein increased mammary cancer, perhaps related to its effect on estrogenic hormone activities,(*45-47*) while its effect on neoplastic(*48*) and pre-neoplastic liver cancer development(*49-51*) was unusually impressive. Dietary animal-based protein at a level of 20% (of total calories) dramatically increased while 5% dietary protein decreased cancer development through the participation of multiple mechanisms acting simultaneously.(*21, 52-55*) None of these nutrient-based effects was attributed to mutations because increased activities were readily reversible, both ways.

In 1975, a seminal conference sponsored by the American Health Foundation(*8*) featured migration patterns (*56, 57*) and time-dependent trends(*58, 59*) on cancer risk, both implicating dietary and environmental causation. A few years later, the U.S. National Academy of Sciences provided funding through the U.S. National Cancer Institute (of NIH), for a committee to undertake a three-year project to review these emerging findings, resulting in a seminal 1982 report,(*4*) *Diet, Nutrition and Cancer* along with a 1983 report(*60*) on suggestions for research. This coincided with the previously cited report on diet and cancer(*3*) that concluded that diet was responsible for a higher proportion of avoidable cancer deaths than even smoking, with estimates that a dietary contribution could be as high as 70%. This same report concluded that genetic predisposition accounted for only 2-4% of avoidable cancer deaths. Thereafter, several institutionally funded summaries of studies on diet and cancer were published over the next quarter century mostly referring to the same conclusion that diet was responsible for about one-third of all cancers.(*5, 61-64*) This meant diets high in animal-based meat, dairy and eggs and low in plant-based vegetables, fruits, whole grains and legumes. Coupled with the laboratory animal-based evidence on the effects of dietary fat and animal based protein on experimental cancer, the human-based evidence suggested that nutrition contributes far more to the cause of cancer than genetics, whether these were genes acquired from previous generations or were created by environmental mutagens.

But, still today, considerable debate and uncertainty exists as to how food affects cancer prevention and treatment, leaving many unanswered questions. Is it the nutrients or is it the 'environmental' chemical carcinogens in food that mostly contribute to human cancer? Are the effects of nutrients consumed in isolation (i.e., supplements) the same as when consumed in food? Because chemical carcinogens mutate genes and nutrients don't, is there any evidence in human studies that genes are more important causes of cancer than nutrition? Is the oft-cited dietary fat association with cancer mortality rates the same for all cancers and is it linear throughout the full range of dietary fat? Does this association depend on type of fat? How does the cancer modifying effect of anti-inflammatory omega-3 fat compare with pro-inflammatory omega-6 fat and are these effects of fat type dependent on total dietary fat? Should nutritional modification of cancer be considered causal? These are only a small sample of such questions that often have a tendency to add more confusion than clarity.

There is reason to believe that there may be more awareness and understanding of the association of diet with heart disease and diabetes during the past couple of centuries than there has been for cancer. But when people transition from simple diets of rural cultures to complex diets of urban cultures, that is, from diets low in fat and protein to diets high in fat and protein, rates of heart disease, diabetes and cancer, as a group, generally increase.(*64, 65*) Is there something to be learned from the causation of these other diseases that might be helpful to an understanding of the causation of cancer, especially something concerning common biochemical mechanisms?

Although nothing much can be specifically attributed to the War on Cancer, it should be noted that, according to the American Cancer Society, recent trends in cancer mortality rates do show some favorable trends since the onset of that program, although it is unclear what role, if any, diet or other aspects of that program has affected these trends.(*66*) Lung cancer is declining in women since about 2000 and in men since about 1990, probably attributed to smoking cessation begun with the Surgeon General's report on smoking in 1964(*67*). Breast cancer has been steadily declining about 2-3% per year in whites and 1-2% per year in blacks, depending on menopausal status. Uterine cancer death rates have been steadily decreasing since about 1930. Stomach cancer started receding several years before 1930, probably in response to the decreasing use of salt preservation of meats in favor of increasing use of food refrigeration. Colorectal cancers began declining in women about 1945 and in men about 1985. Yet, total cancer mortality still is the second leading cause of death in the U.S. Almost one-half of men and somewhat more than one-third of women will be diagnosed with cancer during their lifetimes.(*66*)

Although it is often said that the association of nutrition with cancer is not yet understood, thus diminishing its possible significance, I suggest that existing evidence is more promising than generally known. Consider, for example, the highly variable cancer rates for different countries as a function of diet and other lifestyle practices. If we assume that the lowest observable cancer rate is that which is theoretically achievable and if we were to know the factors causing the higher rates, then all rates above the lowest rate are avoidable. This was suggested by the former director of the UN Agency for Research on Cancer about four decades ago, who stated that 80-90% of total cancer was caused by dietary and environmental factors.(*6*) Based on a similar analysis, a survey of total cancer rates for 65 counties in China showed that 88.5% of male cancers and 80.3% of female cancers are avoidable.(*68*) These estimates of avoidable cancers may be even greater because countries/counties with the lowest rates are likely experiencing the same causal factors observed in the high rate region but at much lower levels of exposure.

Although it is not possible to know what proportion of these avoidable cancers is due to improper nutrition and what proportion is due to environmental chemicals, the evidence strongly suggests that improper nutrition predominates. Among Western type diseases (cancer, heart disease, etc.) in a 65-county, 130 village cohort in rural China, county average serum cholesterol (range of 90-162 mg/dL and mean of 127.6 mg/dL) was the most highly correlated lifestyle factor ($r=0.48$, $p<0.001$) for these diseases (including most cancers(*69, 70*)

Serum cholesterol, substantially influenced by nutritional practices, is correlated with diets having more animal based foods and less plant based foods,(*68*)}an observation that is consistent with experimental animal studies over a century ago.(*71-73*) The popular impression that environmental chemicals are the main dietary cause of cancer—for which there is almost no reliable evidence, diverts attention away from a role for nutrition in cancer.

Diet, Nutrition and Cancer Initiation

Convention among experimental researchers holds that there are three somewhat arbitrary stages of cancer development, initiation, promotion and progression although these stages blend from one stage into the next. The following is presented as initiation but it leads into what also can be considered the beginning of promotion. The effect of nutrition on initiation generally concerns its effect on the formation of mutations from chemical carcinogens, which starts with the enzymatic activation of the carcinogen and concludes with the formation of a mutated cell that is inherited by the cell progeny thereafter. It has long been known that nutrient intakes may substantially alter the amount and the activity of the principle enzyme that activates these carcinogens, the mixed function oxidase that is primarily located in the liver.(*74, 75*) The animal bioassay program(*27*) cited above mostly concerns this step. It attempts to identify environmental chemicals in food (and other environmental settings) that initiate cancer, presumably by mutating normal cells to cancer cells. Mutations mostly occur within regions of the genome that code for the formation of proteins (like enzymes), although there is a growing interest in mutations also acting in non-coding regions.(*76*)

However, some factors participate in cancer development not by mutating genes(*26*) but by acting upon a very complex cellular environment of reactions controlling the expression of these genes. This process is called epigenetics, (*77*) which modifies gene expression in a way that is believed to be heritable, although the responsible mechanisms for this heritable event are not yet fully established. Several participating mechanisms include DNA methylation, histone modification and noncoding RNA participation, as summarized by Ravegnini et al.(*78*) Although there is consensus that epigenetics departs from a rigid association with gene mutations(*77*) that involve changes in DNA base sequence, it still maintains the genetic heritability property. Recently, it has even been suggested,(*77*) that modification of certain epigenetic mechanisms may indicate disease reversal, an observation that suggests something other than heritable change.

One especially informative characteristic of epigenetics is the property of pleiotropy which has been explained by geneticists as "when one gene has an effect on multiple phenotypes" or, perhaps, "as multiple consequences of a single molecular function." This important concept of multiple functions arising from one event also should be applicable to the effects of nutrition on cancer development, involving the effect of one nutrient on

one gene that creates, in turn, multiple phenotypes. For nutrition, however, a broader version of the concept is needed. Foods are composed of a very large number of nutrients and nutrient-like substances, with each nutrient and/or its metabolites affecting multiple genes, and each gene affecting multiple phenotypes. Also, the many foods that we consume create an infinite number of ever changing nutrient-nutrient interactions during digestion, absorption and metabolism, a dynamism of the highest order. A new word, which is more expansive than pleiotropy, is therefore needed that captures this much more expansive breadth of effect. I suggest the word, epitropy. But, unlike the assumption that epigenetics and its pleiotropic characteristic retains the property of heritability, I suggest that nutritional epitropy makes no such claim.

Creating this concept of epitropy without the heritability property is an important departure from the traditional understanding of cancer development, especially when considering the effect of nutrition on development of this disease. That is, there has long been an almost unchallenged enthusiasm that cancer is initiated by a mutation which is subsequently inherited by the cell progeny and which thereafter accumulates a very large number of additional mutations(*79, 80*), especially involving genes that encourage uncontrolled and autonomous cell growth.(*79, 81*) The U.S. National Cancer Institute (of NIH) expresses the dominance of the genetic viewpoint on their website that "cancer is a genetic disease—that is, it is caused by changes to genes that control the way our cells function, especially how they grow and divide."(*82*) This assertion then infers that genes are 'the' main cause of cancer.

However, emphasizing a gene-centric view of cancer has gone too far by excluding other characteristics of cancer development. Focusing on carcinogen-initiated mutations as the primary, perhaps only causal event(s) ignores non-mutagenic causes of human cancer, like the effects of nutrients consumed at inappropriate levels. Dietary protein, for example, when fed at a relatively low level that is adequate for body growth of laboratory rats completely prevented development of pre-cancerous lesions(*83, 84*) and of lifetime tumors(*85*) initiated by one of the most potent of all mutation-inducing carcinogens, aflatoxin. The nutrient effect completely overwhelmed the genetic effect. Similarly, numerous experimental animal studies have shown tumor modifying effects of varied types and amounts of dietary fat (omega-6, omega-3 monounsaturated) and 'calories' on chemical carcinogen-initiated mammary, liver, pancreatic and colon cancers, as reviewed elsewhere.(*4, 64*) If the multiple nutrients known to associate with cancer occurrence, (statically and during migration) are considered, this suggests that nutrition controls gene expression, likely upregulating 'good' genes and downregulating 'bad' genes, as was recently described for the nutritional control of prostate cancer.(*86*) This interpretation is also supported by human population studies which have demonstrated virtually linear, highly significant and biologically plausible correlations of dietary fat(*10*) and animal-based protein(*52*) with certain cancers, regardless of their mutagenic origin. Similarly, diets rich in beta carotene, and other food-based antioxidants and complex carbohydrates, associate with decreasing cancer risk, both in cross-sectional and time-trend studies.(*44*) These nutrient dependent associations and effects question the gene-centric view that mutagenic carcinogens are the primary determinants of human cancer. This is in accord with the previously discussed carcinogen bioassay response where 'proven' mutagenic carcinogens are not sufficiently supported by human observational studies.(*24*)

The uncertainties of interpreting carcinogen response in the bioassay program, the lack of evidence showing significant associations of chemical carcinogens with human cancer in human population studies and the evidence showing nutritional control of gene mutations leading to cancer, renders the animal bioassay as having been very misleading. Focusing on the consumption of foreign mutagenic environmental chemicals as the main cause of human cancer, at the expense of ignoring nutrition that controls post-initiation events, has been unusually costly for more than a half century.

Diet, Nutrition and Cancer Promotion

This second stage of cancer, promotion, traditionally begins with an already mutated cell which clones itself into a multicellular tissue mass that eventually becomes billions, even trillions of cancer cells. The mutated gene acquired during initiation is subsequently inherited by its clone then passed on to its subsequent cell progeny. Cancer 'promotion' in humans may extend over many years (20-30?)(*80*), during which time a large number of mutations are added, possibly giving these cells a selective growth advantage over neighboring normal cells. It has been estimated that there may be 10^8 cells in a human tumor at diagnosis and thousands of mutations per cell, thus billions to trillions of mutations per tumor.(*80*)

Chemicals which promote the growth of these genetically initiated cells are called promoters. But, unlike initiating carcinogens, promoters (at least those initially described)(*87-89*) do not covalently bind to and chemically damage DNA thus do not directly cause classical base pair or base substitution mutations. During much of the early history of the animal bioassay program, for example, there was considerable reluctance to accept promoters as 'real' carcinogens, because they did not cause cancer by initiating mutations. That is, cancer was to be known as a genetic disease, without question.

It is unclear—at least to this writer—at what stage during cancer development does the previously discussed epigenetics begin. Is it at the end of initiation or the beginning of promotion? Or, perhaps, is epigenetics a newer and more detailed description of what was once called promotion? The question of wherein during these three stages epigenetics best fits probably is moot. But it is worth asking because of the proposition that epigenetics is a heritable event, thus tethering it to the initial mutation event and supporting the belief that cancer primarily is a genetic disease.(*90*)

It would seem reasonable to assume that epigenetics is a transition stage between initiation and promotion, thus providing a continuum that connects these stages. More importantly, epigenetics simply describes an exceptionally complex network of events and mechanisms that participate both in gene expression and in cancer promotion. These include a broad spectrum of activities, like the up-regulation of growth promoting genes and the down-regulation of tumor suppressor genes (like p53).(*91*) It may also include a master (driver) gene that controls the killing of unwanted cancer cells, as in apoptosis, itself being another incredibly complex system that discards cells no longer needed. Epigenetics, perceived as genomic instability, may include increased mutation rates(*90, 92, 93*) and the production of mutants with increasing survivability(*90, 94*) Epigenetics may include the constant production and regulation of reactive oxygen species that cause endogenous mutations. It's been estimated that there are 10,000 mutations per cell per day caused by these highly reactive molecules,(*95, 96*) possibly also including mutations occurring in the non-coding regions of the genome.(*76*) Whatever are the mechanisms and domain of epigenetics events, it assumes the addition of many more mutations, from initiation to the

development of a diagnosable cancer, thus maintaining intact the heritability characteristic.

This assumption of heritability is highly consequential because it infers that cancer development is irreversible because back mutations are extremely rare. However, I am skeptical of the irreversibility of cancer, which initially arose from a series of studies in our research laboratory begun almost five decades ago. A research report on experimental animals in India(*48*) had shown that dietary protein had a powerful effect on promoting carcinogen(aflatoxin)-initiated liver cancer. This report coincided with my anecdotal observations in the Philippines suggesting that children of families consuming the most protein (upper socioeconomic class) also were consuming peanuts that were heavily contaminated with the highly mutagenic hepatocarcinogen, aflatoxin (commercial peanut butter(*97*)), suggesting an unusually high susceptibility to primary liver cancer. Subsequently, after more than two dozen in-depth experiments in rats and mice conducted over the next 27-years,(*49-51, 55, 98*) hepatic cancer (hepatocellular carcinoma) initiated by aflatoxin(*99*) grew well with 20% dietary protein (as a percent of calories). But, tumor growth was completely repressed by 5% dietary protein (the requirement for dietary protein by laboratory rats is about 5-6%, recommendation is about 8-10%)(*100*). In retrospect, as I was later to learn, early mutation by the carcinogen aflatoxin did not, singularly, lead to cancer; it had to be promoted by a nutritional stimulus.

Following aflatoxin initiation, dietary protein (at 20% of calories) successively turned on, turned off (5% of calories), turned on (20%) and turned off (5%) tumor development during the first 12 weeks of pre-neoplastic cell growth.(*50, 51*) The same on-off switch also existed during mature tumor development at 40 and 58 weeks of a two-year lifetime study.(*98, 101*) *In the lifetime study,(85, 101) all 60 of the aflatoxin-treated animals fed the 20% protein diet succumbed to liver cancer before the end of the study but all 58 animals initiated with the same dose of aflatoxin but fed the 5% protein diet, were alive and well with no liver cancer.* In short, cancer development was unusually responsive to nutritional exposure, forwards and backwards, at early and late stages of cancer development, presumably by non-mutagenic mutation events.

This dramatic effect of dietary protein (an all-or-none tumor response) occurred within a traditional range of protein consumption (5% to 20% of diet calories), and in the presence of a high dose of mutagen.(*83, 84*) From another vantage point, early cancer initiation by aflatoxin, followed by 5% dietary protein feeding for 9 weeks showed no evidence of early cancer, but these early—but latent—mutations could still be recalled for growth with 20% dietary protein 9-weeks later.(*102*) These and related findings showed that cancer development was completely controlled by dietary protein, even at the highest carcinogen/mutagen exposure and even after a relatively long latency period.

The mechanism for this protein effect was presumably explained by not one but multiple mechanisms occurring both before and after establishment of the initial mutation. High (20%) dietary protein(*103*) increased the mixed function oxidase (MFO) enzyme activity(*104-106*), which activated the initiating carcinogen. As an aside, there seemed to be many foci of initiating mutations observed soon after carcinogen administration because, histologically, isolated clusters of pre-neoplastic clones appeared, each theoretically arising from a mutagenic event.(*49-51*) High dietary protein increased MFO enzyme activity both by increasing enzyme synthesis and by modifying the conformation of this complex enzyme system(*107, 108*) and it did so quickly, even within one day of consumption of protein.(*109*) High dietary protein also increased formation of the covalently bonded adducts of the activated electrophilic carcinogen with the nucleophilic DNA, RNA and nucleoprotein.(*110, 111*)

Beyond the initiation period, low dietary protein also shifted energy (calories) away from its otherwise being used to support cancer growth.(*41, 42*) This shift in calorie utilization results from increased caloric expenditure, both by voluntary exercise (*112, 113*) and by greater caloric expenditure (thermogenesis), as demonstrated by increased oxygen consumption and greater brown adipose tissue activity.(*114, 115*) Low dietary protein also decreases oxygen free radical production,(*116*) which is known to support cancer promotion, (*117, 118*) and depressed natural killer cell activity.(*119*) In parallel animal (mouse) studies, in which hepatitis B virus transfection was the mutagenic agent, the low protein diet decreased circulating levels of growth hormone and insulin like growth factor (IGF-2),(*120*) which otherwise increases cell proliferation and clonal expansion of mutated cells.

Collectively, these multiple mechanisms demonstrated that high dietary protein simultaneously up-regulated mechanisms that increase cancer development and down-regulated the cell's normal ability to reverse such development. This is an excellent demonstration of biological pleiotropy wherein intervention with one nutrient, i.e., animal based protein, simultaneously activates many mechanisms—some up-regulated, some down-regulated—to cause a common effect.

The overall effect of animal-based protein consumption on tumor formation could hardly have been more convincing (0% tumor incidence for the low protein diet up to 100% incidence for the high protein diet). And further, this effect caused by one of the most common proteins consumed, casein, well within traditional ranges of consumption, from just below the recommended intake required for normal function (~8-10% of diet calories) to a level that humans may readily consume (~20% of diet calories).

These experimental animal studies were conducted 20-40 years before the human genome project was undertaken and at a time when methods were not yet available to investigate these events in greater genetic and molecular detail. Modern day methodologies should be expected to elucidate additional mechanisms, all supporting the same response, that is, control of cancer growth by nutrition.

Diet, Nutrition and Cancer Progression

During the third, progression stage of cancer development, cancer cells become more aggressive, advance from their primary site of origin and wander around the body in search of a new home. It has been suggested that only a small number of these circulating cells (<0.02%?)(*121-123*) is able to establish residence in foreign tissue, acquire metabolic independence and wreak havoc in their new surroundings. It is this activity, metastasis, which is responsible for almost all cancer deaths.(*124*) It is also this activity and its corollary development of drug resistance during the progression stage that has generated intense research into finding new ways to target cancer treatment.

Celia-Terrassa and Kang,(*124*) when reviewing the cellular properties of metastasis, pointed out that only a very small fraction of cancer cells circulating in the lymphovascular system are able to successfully adapt to relatively foreign tissue environments. These cells do so by using a network of interrelated mechanisms to create a functional property called cellular plasticity, i.e., adaptability. These mechanisms may include clonal cooperation, epithelial to mesenchymal cell transitions (EMT or its reversal, MET),(*125*) metabolic adaptation, cell proliferation, resistance to apoptosis (controlled cell death), evasion of immune

system attack and resistance to drug therapy, among other mechanisms. A particularly fascinating question concerns how the circulating cancer cell uses its repertoire of aggressive mechanisms to successively counter the multitude of defensive mechanisms that allows residence to take place in foreign tissue. Metastasis is an exceptionally complex system, with countless moving parts. These authors raise the same question that I do as to how will it be possible to target the use of drugs against specific elements of a rapidly changing and complex environment—at least this is my interpretation of their hypothesis.

Nonetheless, these authors seem to remain optimistic. They state that "metastasis initiation may be the culmination of a highly fluid process involving multiple iterations of transitional cellular states, dynamic interactions between clonal populations, and both short-distance and long-range interactions between tumor cells and the host organs," then suggest that simply being aware of this reality still may afford an opportunity that can be "exploited in developing new treatments." Being aware of this reality may be helpful when seeking a drug solution, but negotiating a pathway to that target through an ever-changing complexity (or constellation of targets!), while avoiding unintended side effects and creating imbalance, will be a Herculean challenge that I believe cannot be achieved.

The mechanisms of metastasis represent an extraordinarily complex array of genes, gene mutations and gene expression mechanisms. Methodologies of ever increasing sensitivity and precision are being developed and used to identify mutations and combinations of mutations most critical for developing metastasis—as well as for cancer formation events that precede metastasis.

Very large databases of somatic mutations, including, for example, a catalog of 21,000 genomes or exoms from cancer patients,(*126*) are now available for more discriminating study of an almost infinitely complex system.(*76*) The overarching objective is to define those mutations that best describe the mechanisms of cancer development and, eventually, its deadly metastatic activity in order to develop evidence-based cancer treatment protocols.

Although there are many challenging issues to be resolved when investigating such an enormously complex system, many believe that progress is being made and, by some accounts, it is substantial. For example, studies have identified subgroups of mutations having distinct functions,(*124*) times when mutations occur during the cancer development process, causes of mutations by extrinsic or intrinsic factors,(*127*) mutation derived protein products that may or may not lead to altered amino acid sequences, and mutation outcomes arising from inside or outside of the coding region of the genome, (substantially summarized by Piraino and Furney(*76*)). We also know of driver mutations that are "causally implicated" in the cancer process by conferring "growth advantage"(*128*) to certain cancer cells as they evolve toward metastasis. The number of driver mutations, hence the number of driver genes per cell, seems to be growing(*129*) as the discovery of each new mutation means discovery of yet another function that favors metastatic aggression. This does not necessarily mean the accumulation of new driver mutations per cell on the pathway to metastasis. It may also mean "a greater enrichment of clonal populations"(*124*) of cells already endowed with the needed driver mutations appearing at the primary tumor site. Then, there are the passenger genes with no known function (as of yet) that may be arising by the play of chance during normal cell proliferation.(*124, 128*)

The goals of investigating mutations—their tissue origin, their causes and their effects—are certainly noteworthy and progressive. Such information, according to many researchers, may be crucial to the development of treatment protocols that enable potential drugs to be targeted to the offending events and cell components.(*128*) However—and to repeat—the difficulty of successfully undertaking this task cannot be overemphasized. Perhaps a few quantitative estimates can illustrate the complexity and size of this task [the following citations refer both to the original finding and to the review that cited these observations]. Of the approximately 22,000 protein-encoding genes in the human genome, 350 "show recurrent somatic mutations in cancer, with strong evidence that these contribute to cancer development."(*128, 130*) Mouse studies suggest that "2,000 genes, when appropriately altered, may have potential to contribute to cancer development."(*128, 131*) Then there is the imposing number of mutations per cancer cell, as with the reported 150,000 mutations per cell in adenocarcinoma of the colon.(*80, 90*)

Somatic mutations are highly variable between and within cancer classes, ranging from one to 400,000 mutations per trillion base pairs.(*128, 132*). In 2009, "approximately 100,000 somatic mutations from cancer genomes have been reported in the quarter century since the first somatic mutation was found", with several hundred million more likely to be identified as of this writing.(*128*) Does this exceptionally wide range suggest increased opportunities for discovering unique factors and events for cancer treatment in a way that can be refined and individualized for different tumors and different patients? Is it possible that these powerful genome sequencing and computational methodologies are revealing ever more complex patterns of somatic mutations, some of which may help identify tumor origin, tumor progression, and custom-made, targeted treatment options? Or are these alleged opportunities offset by somatic mutation combinations that work for one type of cancer but not another, making almost insurmountable the development of broad-scope treatment protocols, especially if cancer is considered to be more than 100 distinct diseases, as believed by some?(*128*) Recent research continues to discover yet more complexity, as with the discovery of genetic diversity not only for different tumor types but even for multiple clones of the same tumor.(*133*) Some observers suggest that the future of cancer treatment must be and will be individualized for each patient, possibly relying on the use of multiple pharmaceuticals or polypills, one pill for each dysfunctional event in disease formation.(*134, 135*)

Comprehensive Effects

Ignoring nutrition as a means of cancer control, first proposed around 1800(*136, 137*) has left a trail of undesirable consequences since that time.(*52, 118, 138, 139*)This difficulty, in my opinion, is largely attributable to two parallel histories, 1) a failure to understand the fundamental science of nutrition and 2) an almost unquestioned acceptance of the mutation theory of cancer. Contemporary understanding of nutrition has traditionally relied on investigations of individual nutrient activities, as when they are investigated in isolation in laboratory experiments, in clinical trials in humans and when adjusted for confounding in human observation studies. These are classic examples of reductionism.(*21*) Reductionist investigation of individual nutrient effects certainly produces critically important information but when this information excludes food-based and tissue-based contexts, it can become a source of great confusion. When reductionist information on individual nutrients is aligned with reductionist cancer research on individual gene mutations, both being infinitely complex processes, I believe that a fundamental understanding of the association of nutrition with cancer will

never be possible, even when assuming the increasing power of sophisticated methodologies and computational procedures to sort out complex systems. It is vitally important to acknowledge that these complex systems are not static; they are infinitely dynamic.

It is widely believed that, in addition to our understanding that mutations initiate cancer, there are the uncertain functions of mutations which accumulate in parallel with the rising rates of cancer with age.(*80*) The focus on genes and their mutations throughout the cancer process has intensified even more since completion of the Human Genome Project. Although this project has provided a treasure trove of data for investigating the endless combinations of mutations related to cancer causation and progression, it has been running a very serious risk of overlooking other factors and events that may be even more important than genes and their mutated derivatives. The emphasis given to the mutation theory of cancer continues to rise to new heights, with a recent and highly publicized report showing that cancer incidence for different tissues in our body is highly correlated with the lifetime number of stem cell divisions (r=0.81) when mutations become fixed.(*140*) The greater is the number of cell divisions, the greater is the opportunity for mutations to occur and to be inherited by the cell progeny. These authors claimed that two-thirds of these mutations and their cancer outcomes are random, a play of chance, with no known cause.

This provocative finding(*141*) generated a number of rebuttals(*141-146*) that mostly challenged the idea that cancer is a play of chance that infers a hopeless inability to prevent this disease. These rebuttals also are limited because they, too, assume that mutations are the core events that initiate and sustain cancer development.

The mutation theory of cancer is so firmly established that virtually any new discoveries in cancer research almost reflexively adds new support for this belief. To restate, the U.S. National Cancer Institute of NIH highlights on their website that "cancer is a genetic disease—that is, it is caused by changes to genes that control the way our cells function, especially how they grow and divide."(*82*) In 2013, a prominent international group of cancer researchers whose findings are funded by the highly regarded Wellcome Trust,(*132*) leads with the statement that "all cancers are caused by somatic mutations" and in an earlier 2009 paper by a few of the these same authors,(*128*) the abstract begins by stating that "all cancers arise as a result of changes that have occurred in the DNA sequence of the genomes of cancer cells."

Accepting the prominence of the mutation theory of cancer very likely will continue into the future because of its widespread, mostly unchallenged support. Because of the need to clarify the functions of the several hundred million somatic mutations, the International Cancer Control Consortium (ICGC) is comprehensively characterizing these "genetic events in at least 50 classes of cancer …… requiring high-coverage sequencing of 20,000 cancer genomes or more."(*128*) It is said that "more than 100,000,000,000 base pairs of DNA sequence will probably be required to identify the catalogue of somatic mutations in a single cancer genome."(*128*) These numbers are staggering.

But in spite of this enthusiasm for the future of genetic mutation research as the principle means to understand and control cancer, there are disquieting opinions—voiced from within the genetic mutation research community. It has been recently said that "understanding of the mutational processes that cause somatic mutations in most cancer cases is remarkably limited."(*132*) Also, "the predictive ability and resulting clinical utility of risk evaluation from common genetic variation ….. has to date been found to generally be modest for most multifactorial conditions,"(*147*) although these researchers still believe that refining risk assessment through the use of known genetic mutations will, in the future, warrant clinical application. As this paper was being written, a new paper was published(*148*) with the refreshingly bold title, "Somatic Mutation Theory—Why It's Wrong For Most Cancers", that strongly supports the main message here, namely, that "somatic mutations are epiphenomena or later events occurring after carcinogenesis is already underway" and, further, that a new paradigm for cancer causation is needed. I believe that the hypothesis of this paper focused on a nutrition theory of cancer is this new paradigm.

To be fair to the mutation theory inferences, many authorities believe that establishing mutation locations and functionality in the genome may lead to 1) the clinical application of better, more targeted pharmaceuticals for disease treatment,(*80, 124, 128, 132, 149*) 2) more refined screening of otherwise healthy individuals for future disease(*148, 150-153*) and 3) discovery and control of mutagens in our environment.(*27, 28, 127, 154*) Hope springs eternal for research proposals and clinical applications of this rapidly expanding information. It is said that "…molecular profiling of metastatic tissue [for mutation events] provides invaluable mechanistic insight into the biology underlying metastatic progression and has the potential to identify novel, potentially druggable [sic], drivers of progression."(*151*) Even though there may be "discordance of actionable molecular targets, [it still has] great clinical implication for optimal patient care and the avoidance of unnecessary side effects."(*151*) In other words, cancer will be mainly controlled (treated?) by novel drugs that are targeted to specific genetic events, in spite of divergent observations and an unforgiving complexity of events. When more than one molecular target participates in the final disease outcome and needs to be treated, unique drugs can be combined, as in the proposal for the polypill.(*135, 155*)

Also, the decades old emphasis on environmental mutagens as the main causes of cancer also labors under this reductionist philosophy.(*27*) Newer, more sophisticated methods are making possible the search for genomic imprints (mutation signatures) that reflect past mutagen/carcinogen exposures. This methodology can be useful in assessing the relative contribution of environmental chemicals to overall cancer risk, especially those chemicals that may explain random or 'bad luck' cancers beyond our control,(*140*) thus helping to resolve my criticism of their contribution of total cancer risk . Insight into this question was recently provided by Wu et al,(*127*) who, using a data-driven and model-driven statistical strategy to investigate this claimed relationship of mutations with stem cell divisions,(*140*) concluded that 70-90% of human cancer is derived from extrinsic (environmental) factors that presumably can be controlled. They referred to a few relatively recent epidemiological studies (1988-2012) to support their finding, but they missed a much earlier report by the director of the International Agency for Research on Cancer of the United Nations' World Health Organization who estimated that approximately 90% of all human tumors are influenced by exogenous factors, thus are "theoretically preventable".(*6*) Most of the followup epidemiological studies over the next several years, however, focused on nutritional factors and not on adventitious environmental chemicals as the primary causes of cancer. We need to know how much of the hypothesized "random" cancer can be explained, either by nutritional or by mutagenic factors.

The role of nutrition as a non-mutagenic agent in cancer etiology is, however, mostly ignored if mentioned at all. Alexandrov and 49 co-authors were quick to point out that "cigarette smoke contains over 60 carcinogens"(*156*) and suggested that "it is possible that this complex mixture may initiate other mutational processes."(*132*) But these authors failed to mention the impressive evidence published in 1981 showing

that β-carotene, an indicator of a simple dietary change, is inversely related to lung cancer risk among heavy smokers.(*44*) The incidence of lung cancer, among 2107 male smokers, 40-55 years of age, observed over a 19-year period, was about 85% lower among those with the highest level of β-carotene consumption (comparing highest to lowest quartile), an indication of a remarkable nutritional influence on a disease that is widely thought to result primarily from the onslaught of (*157*)powerful mutagens inhaled in tobacco smoke.(*157*) Many epidemiological studies of the past 40-50 years have shown impressive associations of various cancers with diet and nutrition practices.(*4, 8, 10, 158*) But when and if molecular geneticists cite these studies (very seldom), they mostly assume that mutagens are responsible for these associations.(*159, 160*) Accordingly, nutritional experience, for these authors, is only a 'modifying factor', not a 'cause' that can be manipulated in a way to control cancer.

I believe that the assumption that human cancer is mainly caused by mutations is substantially over-emphasized. I agree that cancer begins with a mutation and, further, that there is convincing evidence that mutations accumulate as cancer advances toward metastasis but this does not mean that cancer is primarily or even solely caused by a series of subsequent mutations. Is it possible that the accumulation of mutations in cancerous tissue represents "epiphenomena or later events occurring after carcinogenesis is already underway?"(*148*) It is very important that the relative contributions of nutrition versus environmental (mutagenic) chemicals to human cancer risk be sorted out. Contemporary research practices, policy development, clinical practice and public knowledge await an answer to this question.

It has long been accepted that a mutation is an extremely rare event, perhaps one mutation occurring every 10^7 cell divisions.(*148*) It also has long been recognized that a back mutation is also a rare event, thus making a forward, then a backward mutation in the same cell an extremely rare event, perhaps 10^{-14}. This suggests that, except for extremely rare occasions, cancer development cannot be reversed. As a consequence, the only way to treat this disease is to kill cancer cells according to Robert Gatenby, as quoted cited by Kaiser,(*161*) especially when these cells, now endowed with thousands of mutations, may have metastasized. This is traditionally accomplished by cytotoxic chemotherapy or radiotherapy, targeted, if possible, to the cancer cells so as to avoid damage to neighboring tissue. More recently, efforts have focused on energizing our immune system to use its innate ability to kill cancer cells, a strategy that may prove to be especially useful for older people whose immune system is in decline.(*162*)

Given this abundant acceptance of the mutation theory of cancer development, I therefore pose a highly provocative question: is it reasonable to hypothesize that cancer, upon diagnosis, can be reversed by nutritional means? If cancer development is dependent on an accumulation of irreversible mutations, the answer to this question must be, "No". Research designed to test this nutrition-reversal-of-cancer hypothesis (undertaken in my laboratory in the early 1980s) showed an opposite answer. It was prompted, in part, by the observations on the effect of smoking on lung cancer risk cited above. It was well established at that time that smoking increases lung cancer risk, presumably due to mutagenic chemicals, and further, that lung cancer risk decreases to baseline within 5-10 years upon cessation of several years of smoking.(*163*) This hinted at the possibility that ongoing disease progression resulting from sustained smoking had been reversed. Additionally, it was also known that lung cancer incidence among heavy smokers decreases almost to that of non-smokers, in a dose response manner, with increased β-carotene consumption (in food).(*44, 157*) Together, these findings suggested that lung cancer risk may be reversed either by quitting smoking and/or (hypothetically) by using a high antioxidant, low animal protein-based diet (i.e., rich in β-carotene). This was the evidence, however valid it was, that led to the laboratory rodent studies on dietary protein and liver cancer mentioned earlier.

The results were striking. Decreasing animal-based protein consumption completely prevented the development of a type of cancer (hepatocellular carcinoma) initiated by the most potent mutagen known at that time (aflatoxin). Also, tumor development could be turned on and off, quite rapidly, simply by increasing then decreasing the consumption of animal-based protein. Although not studied in the same depth, the same on-off switch existed when adjusting dietary fat during the development of experimental pancreatic cancer initiated by azaserine (*164*) and for mammary cancer initiated by dimethylbenzanthracene (DMBA).(*10*) It seems impossible that these nutrition-induced activities could have occurred if cancer development depended on an accumulation of irreversible mutations.

This non-mutagenic nutritional effect (of animal protein) became even more convincing when searching for its explanatory mechanism(s). Not one but several innate, normal mechanisms seem to work simultaneously to control cancer development, some of which enhances and some of which prevents cancer development. In each case, increased consumption of animal protein altered these mechanisms to favor cancer development, elevating mechanisms that promote cancer development and corrupting innate mechanisms that prevent cancer development. The activity of each of nine mechanisms, which was investigated at that time (some evidence being more robust than others), changed in a direction that promotes cancer development, a likelihood of <0.001 (2^{-9} or one chance in 1,024). Now, thirty years later, with much more sophisticated methodology many more mechanisms can be envisioned.

On the likelihood that these animal protein-specific effects on cancer development apply to humans, it is necessary to add the cancer enhancing effects of consuming less whole plant-based foods. Total calorie consumption is mostly a zero-sum game, thus consuming more calorie-containing animal-based protein (as food) means less consumption of whole plant-based foods, which are lower in protein and fat and richer in antioxidants, complex carbohydrates and vitamins. Like the cancer enhancing mechanisms of animal-based protein, similar arrays of cancer repressing mechanisms exist for countless nutrients of plant-based foods. In order to appreciate the full significance of these networks of mechanisms, whether caused by nutrients of animal or plant-based foods, it should be noted that nutrient function, in its totality, is not a simple summation of individual nutrient activities studied in isolation, but the highly dynamic contribution of countless nutrients of whole foods, as described elsewhere.(*54, 139*) The routine and intensely personal desire for animal-based protein has uniquely affected dietary choice ever since its discovery in 1839,(*165, 166*) thus affecting the proportional amounts of animal and plant-based foods in our diets.(*54, 139*) In addition to the pleiotropic effect described here for animal-based protein, the same will be true, albeit in the opposite direction, for multiple plant-based nutrients, especially the phytonutrients of plants that associate with lower cancer risk.

There are many plant substances that exhibit this pleiotropic effect. As summarized elsewhere,(*54, 139*) genistein, which is an anti-cancer estrogen-like isoflavone of soy products,(*167, 168*) engages a large number of mechanisms to produce its effect.(*169*) It modulates genes that regulate cell cycling and programmed cell death (apoptosis), inhibits the nuclear protein complex (nuclear

factor-kappa-B) that activates DNA transcription responsible for stress factor-induced cancer, inhibits transcription and expression of prostate specific antigen that promotes prostate cancer, protects cells against reactive oxygen species (ROS) that encourage cancer growth and blocks estrogen receptors thus minimizing the promotion of breast cancer by endogenous estrogen. Genistein also appears to lower cardiovascular disease risk(*170*) and prevents osteoporosis,(*171*) very likely joining other isoflavones and related components in soy and other legume products that very likely play similar roles with genistein. This is but one of many plant-based substances that employs pleiotropy to lower breast cancer risk while engaging related soy phytochemicals to minimize risks for cardiovascular disease and osteoporosis, as in epitropy.

Another example of a potential cancer preventive agent for prostate cancer is lycopene, a red carotenoid pigment in tomatoes, which has strong antioxidant activity and which is hypothesized to prevent prostate cancer and its predecessor, benign prostatic hyperplasia,(*172*) although this evidence is not convincing when lycopene is consumed as a supplement.(*173*) As an antioxidant, lycopene induces several mechanisms that favor cancer inhibition, including anti-proliferative, anti-oxidative, and anti-inflammatory responses, while down-regulating genes contributing to the androgen receptor signaling pathway,(*174*) all of which illustrate pleiotropy.

Many plant nutrients and related phytochemicals are well known antioxidants that counterbalance the oxidative stress largely created by reactive oxygen species (ROS). As brilliantly summarized by Reuter et al,(*175*) reactive oxygen species are endogenously produced in the body, mostly during mitochondrial respiration, and they play a vital physiological role, as in their attack and destruction of invasive disease producing organisms. However, it is also important that ROS activities be kept in check, that is, the dynamic production and removal of ROS represents a pro-oxidant/anti-oxidant system that requires exquisite homeostatic control. Prolonged tissue excess of these highly reactive molecules leads to chronic inflammation that encourages aging and associated chronic diseases like cancer, cardiovascular disease and diabetes, among others. The breadth of effect of this infinitely complex system cannot be overemphasized and food choice plays a vital role in keeping this oxidant-antioxidant system in balance. This pro-oxidant/anti-oxidant system distinguishes the relative contributions of whole plant based foods (rich in antioxidants) from their counterparts, foods that are highly processed and/or of animal origin (often rich in promoting the production of ROS).

Within the context discussed here, the ROS system illustrates both the pleiotropy and epitropy concepts of nutrient function. “Oxidative stress can activate a variety of transcription factors that lead to the expression of over 500 different genes, including those for growth factors, inflammatory cytokines, chemokines, cell cycle regulatory molecules and anti-inflammatory molecules.”(*175*) Antioxidants, which represent a very large and growing number of phytochemicals from plants (e.g., lycopene, β-carotene, cryptoxanthin, canthaxanthin and a few hundred other carotenoids, tocopherols like the vitamin E analogs, genistein and other isoflavones), combine their activities upon consumption to upregulate families of genes to produce even larger families of protein products (enzymes) that prevent and/or reverse families of diseases (e.g., cancer, aging, cardiovascular, diabetes, neurological, autoimmune). In this case, there are pro- and anti-oxidant members of these contrasting families that illustrate the relative nutritional properties of whole plant-based foods from animal based foods (and highly processed food fragments). Both groups involve an exceptionally large number of substances, reactions and activities and it is this complexity and this contrast which must be acknowledged in any cause-and-effect hypothesis.

The relative proportions of disease risk specifically attributable to the direct effects of animal-based protein (as food) compared to the effects of declining consumption of whole plant-based foods, however, is difficult to assess. Almost no human studies—except for a very few studies on heart disease(*176-178*) and prostate cancer(*86*)—have included individuals using a whole food plant-based (WFPB) diet with little or no added fat and refined carbohydrates. Findings of occasional studies on self-proclaimed vegetarians and vegans as experimental subjects certainly are indicative of these effects but they are not especially relevant for fully assessing the effects of a WFPB diet. Most vegetarians consume generous amounts of dairy and some eggs and fish. Vegans, although consuming no animal-based protein, still consume substantial amounts of fat(*179*) and refined carbohydrates. The largest survey of these ‘V’ diets (803 adult vegans, average total dietary fat of 30.4% for males and 30.5% for females; 6673 adult vegetarians, average total dietary fat of 30.0% for males and 29.9% for females) showed a remarkable similarity with 18,244 adult meat eaters (30.9% for males and 31.4% for females).(*179, 180*) Similarly, consumption of total sugars was within the narrow range of 22.6% to 24.6% for both sexes and all three diets. Thus, it is important to emphasize that findings on vegan and vegetarian diets are not the same as that desired for the WFPD diet (about 9-12% total dietary fat and little or no sugar).(*55, 176, 177, 181, 182*)

These findings on the apparent effects of animal-based protein on cancer development, as observed in correlation studies on humans, become much more significant when nutritional function is viewed as the comprehensive, epitropic effects of virtually countless nutrients, nutrient-like substances and their interactions during their consumption, digestion, absorption and metabolic disposition. Additional evidence for a comprehensive nutrition-based effect on reversal of chronic, degenerative disease (not obviously mutagenic) is the evidence showing the ability of a whole food plant based diet in intervention studies to dramatically reverse heart disease.(*176, 177, 183*) Esselstyn et al, in a recent report(*178, 184*) of 198 patients with confirmed heart disease, found that 89% were compliant with his initial advice given in a 5-hour session to consume a whole food plant based diet. After 2-7 years of follow-up, compliant patients suffered a recurrence rate of cardiac events of <1% while non-compliant patients suffered a recurrence rate of 62%. Similar results were earlier reported by Ornish et al at one(*176*) and five years(*185, 186*) after onset of treatment. That study, which also included stress management and exercise(*176*), was one of the first(*183*) to publish peer-reviewed findings on the effect of the whole food plant-based on cardiovascular disease. The etiology of heart disease is not like that for cancer because it has not yet been shown to be the result of mutations but, like cancer, it is a degenerative multifactorial disease forming over many years, also involving a similarly comprehensive effect of nutrition.

On the future of cancer research and its dependency on public support, the previously cited exorbitant costs of developing and marketing new cancer drugs,(*187*) the widely known side effects of chemotherapy,(*188*) the marketplace conditions that “inadvertently incentivize the pursuit of marginal outcomes” and the average $171,000 per year per patient cost for cancer drug treatment(*189*) are factors that compromise public support. I am very much aware of the recent promise and promotion of immunotherapy as a ‘new way’ to think about cancer treatment along with a few welcome reports of individual patient successes. I am also aware of the fascinating discoveries tethered to genetic

function and the many ways that it might complement newer treatment modalities. But these 'modern medicine' highlights still rely on searching for treatments which selectively target cancer cells enmeshed within an incomprehensibly complex maze of reactions established and controlled for eons of time by a natural order of things.

I submit that what has been done and what is still being planned for future cancer treatment protocols is over-simplistic, relatively ineffective, extremely costly and likely to be encumbered with unintended and counterproductive side effects. Such a strategy is over-simplistic because it ignores the infinitely complex biology that underlies, respectively, cancer development and nutritional function. Cancer cannot primarily be the consequence of a series of mutations. Back-mutation of mutated cells to normalcy is too improbable. Yet, experimental animal evidence shows that cancer development can be reversed by nutritional means. Nutrition, in turn, cannot be ascribed to the effects of individual nutrients operating independently and in isolation because single nutrient supplements do not faithfully mimic their biological activities in food. That is, these combined observations, each representing infinitely complex but still highly integrated phenomena, support the hypothesis that cancer is primarily a nutrition-responsive disease. These phenomena, considered independently or together, represent what might be called Nature.

There is only one way to affirm or deny this hypothesis and this is to conduct an intervention trial in human cancer patients. Evidence is already available showing that a whole food plant-based diet reverses heart disease remarkably effectively while similar but less robust evidence supports the same effect on other chronic illnesses. Given the evidence showing that cancer could be considered primarily a nutrition-based disease, instead of a genetic disease, it is therefore reasonable to assume that the same nutritional effect on cancer may also exist. It should be noted, however, that an association of diet and nutrition with cancer (or other disease outcomes) is far more than a single nutrient functioning through a single mechanism that produces a single outcome. It is time that we recognize the complexity of these systems, then use this information to chart a more efficacious pathway to future cancer care practices which, not so incidentally, are similar to those for other illnesses as well.

Funding Support

NCI/NIH Grants R01 CA20079 and NCI/NIH R01 CA34205; NIEHS/NIH R01 ES00336, R01 ES00380, R01 ES00256 and R01 ES00541; USPHS P01 CA26755, P01 CA26755 and T2E50705A

Acknowledgements

I acknowledge the many graduate students, post-doctoral fellows, visiting faculty and other colleagues in my laboratory whose experimental research on the many parts—unknowing of the comprehensiveness of 'whole' story at the time—made this account possible. I also acknowledge four clinicians whose work with patients greatly strengthened the results that we obtained in the laboratory. Alphabetically, they are Caldwell Esselstyn, Jr. MD at the Cleveland Clinic, Alan Goldhamer, DC, at the Truth North Clinic in Santa Rosa CA, John McDougall MD at his private clinic in Santa Rosa CA and Dean Ornish MD at the University of California San Francisco.

1. K. Rezvani, R. Rouce, E. Liu, E. Shpall, Engineering Natural Killer Cells for Cancer Immunotherapy. *Mol Ther*, (2017).
2. G. Morgan, R. Ward, M. Barton, The contribution of cytotoxic chemotherapy to 5-year survival in adult malignancies. *Clin. Oncol. (R. Coll. Radiol.)* **16**, 549-560 (2004).
3. R. Doll, R. Peto, The causes of cancer: quantitative estimates of avoidable risks of cancer in the Unites States today. *J Natl Cancer Inst* **66**, 1191-1308 (1981).
4. Committee on Diet Nutrition and Cancer, *Diet, Nutrition and Cancer*. (National Academy Press, Washington, DC, 1982), pp. 478.
5. Expert Panel, *Food, nutrition and the prevention of cancer, a global perspective*. (American Institute for Cancer Research/World Cancer Research Fund, Washington, D.C., 1997), pp. 670.
6. J. Higginson, Present trends in cancer epidemiology. *Proc. Can. Cancer Conf.* **8**, 40-75 (1969).
7. B. S. Drasar, D. Irving, Environmental factors and cancer of the colon and breast. *Br. J. Cancer* **27**, 167-172 (1973).
8. E. L. Wynder, The epidemiogy of large bowel cancer. *Cancer Res.* **35**, 3388-3394 (1975).
9. K. K. Carroll, H. T. Khor, in *Progress in Biochemical Pharmacology: Lipids and Tumors*. (S. Karger, New York, 1975), vol. 10, pp. 308-345.
10. K. K. Carroll, L. M. Braden, J. A. Bell, R. Kalamegham, Fat and cancer. *Cancer* **58**, 1818-1825 (1986).
11. L. A. Cohen, Diet and cancer. *Sci. Am.* **257**, 42-48 (1987).
12. D. Armstrong, R. Doll, Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. *Int. J. Cancer* **15**, 617-631 (1975).
13. E. L. Wynder, D. P. Rose, Diet and breast cancer. *Hosp Pract (Off Ed)* **19**, 73-78, 83-78 (1984).
14. L. A. Cohen, P. C. Chan, E. L. Wynder, The role of a high-fat diet in enhancing the development of mammary tumors in ovariectomized rats. *Cancer* **47**, 66-71 (1981).
15. S. A. Bingham *et al.*, Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study. *Lancet* **361**, 1496-1501 (2003).
16. E. L. Wynder, B. S. Reddy, Metabolic epidemiology of colorectal cancer. *Cancer* **34**, 801-805 (1974).
17. S. Bingham, D. R. Williams, T. J. Cole, W. P. James, Dietary fibre and regional large-bowel cancer mortality in Britain. *Br J Cancer* **40**, 456-463 (1979).
18. D. P. Burkitt, Epidemiology of cancer of the colon and the rectum. *Cancer* **28**, 3-13 (1971).
19. M. Tortorello, The great cranberry scare. *The New Yorker*. 2015.
20. T. C. Campbell, Chemical carcinogens and human risk assessment. *Fed. Proc.* **39**, 2467-2484 (1980).
21. T. C. Campbell, *Whole. Rethinking the science of nutrition (with H. Jacobson)*. (BenBella Books, Dallas TX, 2013), pp. 328.
22. G. Kroll, The "Silent Springs" of Rachel Carson: mass media and origins of modern environmentalism. *Public Underst. Sci.* **10**, 403-410 (2001).
23. J. H. Weisburger, E. K. Weisburger, in *Methods in cancer research,* H. Busch, Ed. (Academic Press, New York, 1967), vol. 1, pp. 307-398.
24. D. G. Hoel, D. W. Gaylor, R. L. Kirschstein, U. Saffiotti, M. A. Schneiderman, Estimation of risks of irreversible, delayed toxicity. *J Toxicol Environ Health* **1**, 133-151 (1975).
25. E. K. Weisburger, History of the Bioassay Program of the National Cancer Institute. *Prog Exp Tumor Res* **26**, 187-201 (1983).
26. A. C. Jacobs, K. P. Hatfield, History of chronic toxicity and animal carcinogenicity studies for pharmaceuticals. *Vet Pathol* **50**, 324-333 (2013).
27. National Toxicology Program, Report on carcinogens. 2011.
28. D. P. Rall, Difficulties in extrapolating the results of toxicity studies in laboratory animals to man. *Environ Res* **2**, 360-367 (1969).
29. E. Boyland, The correlation of experimental carcinogenesis and cancer in man. *Prog. Exp. Tumor Res.* **11**, 222-234 (1967).

30. J. Huff, M. F. Jacobson, D. L. Davis, The limits of two-year bioassay exposure regimens for identifying chemical carcinogens. *Environ. Health Perspect.* 2008.
31. S. M. Cohen, Human carcinogenic risk evaluation: an alternative approach to the two-year rodent bioassay. *Toxicol. Sci.* 2004.
32. T. C. Campbell, A decision tree approach to the regulation of food chemicals associated with irreversible toxicities. *Regulatory Tox. Pharm.* **1**, (1981).
33. P. Shubik, Opinion: the validity of animal studies with chemical carcinogens. *CA Cancer J Clin* **31**, 120-123 (1981).
34. B. Ames, Dietary carcinogens and anticarcinogens. *Sci.* **221**, 1256-1264 (1983).
35. J. McCann, E. Choi, E. Yamasaki, B. N. Ames, Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals. *Proc. Natl. Acad. Sci., U.S.A.* **72**, 5135-5139 (1975).
36. D. P. Rose, A. P. Boyar, L. Cohen, L. E. Strong, Effect of a low-fat diet on hormone levels in women with cystic breast disease. I. Serum steroids and gonadotropins. *J. Natl. Cancer Inst.* **78**, 623-626 (1987).
37. D. P. Rose, A. P. Boyar, K. Kettunen, Diet, serum breast fluid growth hormone and prolactin levels in normal premenopausal Finnish and American women. *Nutrition and Cancer* **11**, 179-188 (1988).
38. D. P. Rose, Dietary fiber and breast cancer. *Nutrition and Cancer* **13**, 1-8 (1990).
39. D. P. Rose, M. Goldman, J. M. Connolly, L. E. Strong, High-fiber diet reduces serum estrogen concentrations in premenopausal women. *Am. J. Clin. Nutr.* **54**, 520-525 (1991).
40. B. S. Reddy, E. L. Wynder, Large bowel carcinogenesis: fecal constituents of populations with disease incidence rates of colon cancer. *J. Nat. Cancer Inst.* **50**, 1437-1442 (1973).
41. D. Kritchevsky, Calories and chemically induced tumors in rodents. *Comprehensive Therapy* **11**, 35-39 (1985).
42. D. Kritchevsky, Caloric restriction and cancer. *J. Nutr. Sci. Vitaminol* **47**, 13-19 (2001).
43. M. B. Sporn, N. M. Dunlop, D. L. Newton, J. M. Smith, Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids). *Fed. Proc.* **35**, 1332-1338 (1976).
44. R. Peto, R. Doll, J. D. Buckley, Can dietary beta-carotene materially reduce human cancer rates? *Nature* **290**, 201-208 (1981).
45. E. J. Hawrylewicz, H. H. Huang, J. Liu, Dietary protein enhancement of N-nitroso-methylurea-induced mammary carcinogenesis, and their effect on hormone regulation in rats. *Cancer Res.* **46**, 4395-4399 (1986).
46. H. H. Huang, E. J. Hawrylewicz, J. Q. Kissane, E. A. Drab, Effect of protein diet on release of prolactin and ovarian steroids in female rats. *Nutrition Reports International* **26**, 807-820 (1982).
47. M. C. A. Sanz, J.-M. Liu, H. H. Huang, E. J. Hawrylewicz, Effect of dietary protein on morphoplogic development of rat mammary gland. *J. Natl. Cancer Inst.* **77**, 477-487 (1986).
48. T. V. Madhavan, C. Gopalan, The effect of dietary protein on carcinogenesis of aflatoxin. *Arch. Path.* **85**, 133-137 (1968).
49. B. S. Appleton, T. C. Campbell, Effect of high and low dietary protein on the dosing and postdosing periods of aflatoxin B1-induced hepatic preneoplastic lesion development in the rat. *Cancer Res.* **43**, 2150-2154 (1983).
50. B. S. Appleton, T. C. Campbell, Dietary protein intervention during the post-dosing phase of aflatoxin B1-induced hepatic preneoplastic lesion development. *J. Natl. Cancer Inst.* **70**, 547-549 (1983).
51. B. S. Appleton, T. C. Campbell, Inhibition of aflatoxin-initiated preneoplastic liver lesions by low dietary protein. *Nutrition and Cancer* **3**, 200-206 (1982).
52. T. C. Campbell, Untold nutrition. *Nutrition and Cancer* **66**, 1077-1082 (2014).
53. T. C. Campbell, A plant based diet and animal protein: questioning dietary fat and considering animal protein as the main cause of heart disease. *J. Geriatric Cardiol.* **14**, 331-337 (2017).
54. T. C. Campbell, Nutrition renaissance and public health policy. *Journ. Nutr. Biology* **In press**, (2017).
55. T. C. Campbell, T. M. Campbell, II, *The China Study, Startling Implications for Diet, Weight Loss, and Long-Term Health*. (BenBella Books, Inc., Dallas, TX, 2005), pp. 417.
56. W. Haenszel, Studies of migrant populations. *J. Chronic Dis* **23**, 289-291 (1970).
57. W. Haenszel, M. Kurihara, Studies of Japanese Migrants: mortality from cancer and other disease among Japanese and the United States. *J Natl Cancer Inst* **40**, 43-68 (1968).
58. E. L. Wynder, G. B. Gori, Contribution of the environment to cancer incidence: an epidemiologic exercise. *J. Natl. Cancer Inst.* **58**, 825-832 (1977).
59. C. P. Howson, T. Hiyama, E. L. Wynder, The decline in gastric cancer: Epidemiology of an unplanned triumph. *Epidemiol. Rev.* **8**, 1-2700000 (1986).
60. Committee on Diet Nutrition and Cancer, *Diet, nutrition and cancer: directions for research*. N. A. o. S. National Research Council, Ed., (National Academy Press, Washington, D.C., 1983), pp. 74.
61. World Cancer Research Fund/American Institute for Cancer Research, "Food, Nutrition, Physical Activity, and Prevention of Cancer: A Global Perspective," (American Institute for Cancer Research, Washington, D.C>, 2007).
62. World Health Organization, "Obesity, preventing and managing the global epidemic: report of the WHO consultation of obesity," (World Health Organization, Geneva, 1997).
63. U.S. Department of Health and Human Services and U.S. Department of Agriculture, *Nutrition monitoring in the United States: a progress report from the Joint Nutrition Monitoring Evaluation Committee. DHHS publication no. (PHS) 86-1255*. (National Center for Health Statistics, Hyattsville, MD, 1986).
64. National Research Council, Committee on Diet and Health, *Diet and health: implications for reducing chronic disease risk.*, (National Academy Press, Washington, D.C., 1989), pp. 749.
65. United States Department of Health and Human Services, *The Surgeon General's Report on Nutrition and Health*. D. P. P. N.-. Public Health Service, Ed., (Superintendent of Documents, U.S. Government Printing Office, Washington, D.C., 1988), pp. 727.
66. American Cancer Society, "Cancer facts and figures 2015," (Atlanta, GA, 2015).
67. *Smoking and heallth: report of the advisory committee to the Surgeon General of the Public Health* (1964).
68. J. Chen, T. C. Campbell, J. Li, R. Peto, *Diet, life-style and mortality in China. A study of the characteristics of 65 Chinese counties*. (Oxford University Press; Cornell University Press; People's Medical Publishing House, Oxford, UK; Ithaca, NY; Beijing, PRC, 1990), pp. 894.
69. T. C. Campbell *et al.*, China: from diseases of poverty to diseases of affluence. Policy implications of the epidemiological transition. *Ecology of Food and Nutrition* **27**, 133-144 (1992).
70. T. C. Campbell, J. Chen, C. Liu, J. Li, B. Parpia, Non-association of aflatoxin with primary liver cancer in a cross-sectional ecologic survey in the People's Republic of China. *Cancer Res.* **50**, 6882-6893 (1990).
71. A. Ignatowski, Uber die Wirbung des tierischen eiweiss auf die aorta und die parenchymatosen organe der kaninchen. *Vrichows Arch Pathol Anat Physiol Klin Med* **198**, 248-270 (1909).
72. D. Kritchevsky, S. K. Czarnecki, in *Animal and vegetable proteins in lipid metabolism and atherosclerosis,* M. J. Gibney, D. Kritchevsky, Eds. (Alan R. Liss, Inc., New York, 1983), vol. 8, pp. 1-7.
73. L. H. Newburgh, S. Clarkson, The relation between atherosclerosis and ingested cholesterol in the rabbit. *J. Exp. Med.* **43**, 595-612 (1926).
74. T. C. Campbell, J. R. Hayes, The effect of quantity and quality of dietary protein on drug metabolism. *Fed. Proc.* **35**, 2470-2474 (1976).
75. T. C. Campbell, J. R. Hayes, Role of nutrition in the drug metabolizing system. *Pharmacol. Revs.* **26**, 171-197 (1974).
76. S. W. Piraino, S. J. Furney, Beyond the exome: the role of non-coding somatic mutations in cancer. *Ann Oncol*, (2015).
77. D. Bhattacharjee, S. Shenoy, K. L. Bairy, DNA methylation and chromatin remodeling: the blueprint of cancer epidenetics. *Scientifica* **2016:6072357**, (2016).
78. G. Ravegnini, G. Sammarini, P. Hrelia, S. Angelini, Key Genetic and Epigenetic Mechanisms in Chemical Carcinogenesis. *Toxicol Sci* **148**, 2-13 (2015).

79. J. H. Bielas, K. R. Loeb, B. P. Rubin, L. D. True, L. A. Loeb, Human cancers express a mutator phenotype. *Proc Natl Acad Sci U S A* **103**, 18238-18242 (2006).
80. L. A. Loeb, K. R. Loeb, J. P. Anderson, Multiple mutations and cancer. *Proc Natl Acad Sci U S A* **100**, 776-781 (2003).
81. T. Sjoblom *et al.*, The consensus coding sequences of human breast and colorectal cancers. *Science* **314**, 268-274 (2006).
82. http://www.cancer.gov/about-cancer/what-is-cancer, What is cancer? 2016.
83. G. E. Dunaif, T. C. Campbell, Relative contribution of dietary protein level and aflatoxin B1 dose in generation of presumptive preneoplastic foci in rat liver. *J Natl Cancer Inst* **78**, 365-369 (1987).
84. G. E. Dunaif, T. C. Campbell, Dietary protein level and aflatoxin B1-induced preneoplastic hepatic lesions in the rat. *Journal of Nutrition* **117**, 1298-1302 (1987).
85. L. D. Youngman, T. C. Campbell, Inhibition of aflatoxin B1-induced gamma-glutamyl transpeptidase positive (GGT+) hepatic preneoplastic foci and tumors by low protein diets: evidence that altered GGT+ foci indicate neoplastic potential. *Carcinogenesis* **13**, 1607-1613 (1992).
86. D. Ornish *et al.*, Effect of comprehensive lifestyle changes on telomerase activity and telomere length in men with biopsy-proven low-risk prostate cancer: 5-year follow-up of a descriptive pilot study. *Lancet Oncol.* **14**, 1112-1120 (2013).
87. I. Berenblum, A speculative review: the probable nature of promoting action and its significance in the understanding of the mechanism of carcinogenesis. *Cancer Res.* **14**, 471-477 (1954).
88. I. Berenblum, The cocarcinogenic action of croton resin. *Cancer Res.* **1**, 44-48 (1941).
89. I. Berenblum, P. Shubik, The persistence of latent tumour cells induced in the mouse's skin by a single application of 9:10-dimethyl-1:2-benzanthracene. *Brit. J. Cancer* **3**, 384-386 (1949).
90. I. Tomlinson, W. Bodmer, Selection, the mutation rate and cancer: ensuring that the tail does not wag the dog. *Nature medicine* **5**, 11-12 (1999).
91. J. S. Fridman, S. W. Lowe, Control of apoptosis by p53. *Oncogene* **22**, 9030-9040 (2003).
92. L. A. Loeb, C. F. Springgate, N. Battula, Errors in DNA replication as a basis of malignant changes. *Cancer Res* **34**, 2311-2321 (1974).
93. L. A. Loeb, Transient expression of a mutator phenotype in cancer cells. *Science* **277**, 1449-1450 (1997).
94. P. C. Nowell, The clonal evolution of tumor cell populations. *Science* **194**, 23-28 (1976).
95. T. M. Reid, L. A. Loeb, Mutagenic specificity of oxygen radicals produced by human leukemia cells. *Cancer Res* **52**, 1082-1086 (1992).
96. B. N. Ames, L. S. Gold, W. Willett, C., The causes and prevention of cancer. *Proc. National Acad. Sci* **92**, 5258-5256 (1995).
97. T. C. Campbell, Present day knowledge on aflatoxin. *Philadelphia Journal of Nutrition* **20**, 193-201 (1967).
98. L. D. Youngman, The sustained development of preneoplastic lesions depends on high protein diets. *Nutrition and Cancer* **18**, 131-142 (1992).
99. G. N. Wogan, S. Paglialunga, P. M. Newberne, Carcinogenic effects of low dietary levels of aflatoxin B_1 in rats. *Food Cosmet. Toxicol.* **12**, 681-685 (1974).
100. Subcommittee on Laboratory Animal Nutrition, *Nutrient requirements of laboratory animals. Second revised edition, number 10.*, (National Academy Press, Washington, D.C., 1972), pp. 116.
101. L. D. Youngman, *The growth and development of aflatoxin B1-induced preneoplastic lesions, tumors, metastasis, and spontaneous tumors as they are influenced by dietary protein level, type, and intervention.*, (Cornell University, Ph.D. Thesis, Ithaca, NY, 1990), pp. 203.
102. L. D. Youngman, T. C. Campbell, High protein intake promotes the growth of preneoplastic foci in Fischer #344 rats: evidence that early reemodeled foci retain the potential for future growth. *Journal of Nutrition* **121**, 1454-1461 (1991).
103. A. Kato, T. Oshima, K. Tomizawa, Toxicity and metabolism of drugs in relation to dietary protein. *Jap. J. Pharmacol.* **18**, 356-366 (1968).
104. M. U. K. Mgbodile, T. C. Campbell, Effect of protein deprivation of male weanling rats on the kinetics of hepatic microsomal enzyme activity. *Journal of Nutrition* **102**, 53-60 (1972).
105. M. U. K. Mgbodile, J. R. Hayes, T. C. Campbell, Effect of protein deficiency on the inducibility of the hepatic microsomal drug-metabolizing enzyme system. II. Effect on enzyme kinetics and electron transport system. *Biochem. Pharmacol.* **22**, 1125-1132 (1973).
106. J. R. Hayes, M. U. K. Mgbodile, T. C. Campbell, Effect of protein deficiency on the inducibility of the hepatic microsomal drug-metabolizing enzyme system. I. Effect on substrate interaction with cytochrome P-450. *Biochem. Pharmacol.* **22**, 1005-1014 (1973).
107. H. L. Gurtoo, T. C. Campbell, A kinetic approach to a study of the induction of rat liver microsomal hydroxylase after pretreatment with 3,4-benzpyrene and aflatoxin B_1. *Biochem. Pharmacol.* **19**, 1729-1735 (1970).
108. L. S. Nerurkar, J. R. Hayes, T. C. Campbell, The reconstitution of hepatic microsomal mixed function oxidase activity with fractions derived from weanling rats fed different levels of protein. *Journal of Nutrition* **108**, 678-686 (1978).
109. M. Maso, Undergraduate Honors Thesis, Cornell University, Ithaca, NY (1979).
110. R. S. Preston, J. R. Hayes, T. C. Campbell, The effect of protein deficiency on the in vivo binding of aflatoxin B_1 to rat liver macromolecules. *Life Sci.* **19**, 1191-1198 (1976).
111. L. O. Prince, T. C. Campbell, Effects of sex difference and dietary protein level on the binding of aflatoxin B_1 to rat liver chromatin proteins in vivo. *Cancer Res.* **42**, 5053-5059 (1982).
112. E. Krieger, Undergraduate Honors Dissertation, Cornell University, Ithaca, NY (1988).
113. E. Krieger, L. D. Youngman, T. C. Campbell, The modulation of aflatoxin(AFB1) induced preneoplastic lesions by dietary protein and voluntary exercise in Fischer 344 rats. *FASEB J.* **2**, 3304 Abs. (1988).
114. F. Horio, L. D. Youngman, R. C. Bell, T. C. Campbell, Thermogenesis, low-protein diets, and decreased development of AFB1-induced preneoplastic foci in rat liver. *Nutrition and Cancer* **16**, 31-41 (1991).
115. R. C. Bell, D. A. Levitsky, T. C. Campbell, Enhanced thermogenesis and reduced growth rates do not inhibit GGT+ hepatic preneoplastic foci development. *FASEB J.* **6**, 1395 Abs (1992).
116. L. D. Youngman, J. Y. Park, B. N. Ames, Protein oxidation associated with aging is reduced by dietary restriction of protein or calories. *Proc. National Acad. Sci* **89**, 9112-9116 (1992).
117. K. K. Carroll, Lipid and carcinogenesis. *Prog. Clin. Biol. Res.* **206**, 237-244 (1986).
118. K. K. Carroll, in *Dietary Fat and Cancer,* C. Ip, D. F. Birt, A. E. Rogers, C. Mettlin, Eds. (Alan R. Liss, Inc., 1986), pp. 231-248.
119. R. C. Bell, K. A. Golemboski, R. R. Dietert, T. C. Campbell, Long-term intake of a low-casein diet is associated with higher relative NK cell cytotoxic activity in F344 rats. *Nutrition and Cancer* **22**, 151-162 (1994).
120. J. Hu *et al.*, Repression of hepatitis B virus (HBV) transgene and HBV-induced liver injury by low protein diet. *Oncogene* **15**, 2795-2801 (1997).
121. K. J. Luzzi *et al.*, Multistep nature of metastatic inefficiency: dormancy of solitary cells after successful extravasation and limited survival of early micrometastases. *Am J Pathol* **153**, 865-873 (1998).
122. M. D. Cameron *et al.*, Temporal progression of metastasis in lung: cell survival, dormancy, and location dependence of metastatic inefficiency. *Cancer Res* **60**, 2541-2546 (2000).
123. A. F. Chambers, A. C. Groom, I. C. MacDonald, Dissemination and growth of cancer cells in metastatic sites. *Nat Rev Cancer* **2**, 563-572 (2002).
124. T. Celia-Terrassa, Y. Kang, Distinctive properties of metastasis-initiating cells. *Genes Dev* **30**, 892-908 (2016).
125. J. P. Thiery, H. Acloque, R. Y. Huang, M. A. Nieto, Epithelial-mesenchymal transitions in development and disease. *Cell* **139**, 871-890 (2009).
126. S. A. Forbes *et al.*, COSMIC: exploring the world's knowledge of somatic mutations in human cancer. *Nucleic Acids Res* **43**, D805-811 (2015).

127. S. Wu, S. Powers, W. Zhu, Y. A. Hannun, Substantial contribution of extrinsic risk factors to cancer development. *Nature* **529**, 43-47 (2016).
128. M. R. Stratton, P. J. Campbell, P. A. Futreal, The cancer genome. *Nature* **458.7239**, 719-724 (2009).
129. N. Beerenwinkel *et al.*, Genetic progression and the waiting time to cancer. *PLoS Comput Biol* **3**, e225 (2007).
130. P. A. Futreal *et al.*, A census of human cancer genes. *Nat Rev Cancer* **4**, 177-183 (2004).
131. I. P. Touw, S. J. Erkeland, Retroviral insertion mutagenesis in mice as a comparative oncogenomics tool to identify disease genes in human leukemia. *Mol Ther* **15**, 13-19 (2007).
132. L. B. Alexandrov *et al.*, Signatures of mutational processes in human cancer. *Nature* **500**, 415-421 (2013).
133. D. Ryu, J. G. Joung, N. K. Kim, K. T. Kim, W. Y. Park, Deciphering intratumor heterogeneity using cancer genome analysis. *Human genetics* **135**, 635-642 (2016).
134. N. J. Wald, M. R. Law, A strategy to reduce cardiovascular disease by more than 80%. *Brit. Med. Journ.* **326**, 1419-1424 (2003).
135. J. M. Castellano, G. Sanz, V. Fuster, Evolution of the polypill concept and ongoing clinical trials. *Can J Cardiol* **30**, 520-526 (2014).
136. W. Lambe, *Additional reports on the effects of a peculiar regimen in cases of cancer, scrofula, consumption, asthma, and other chronic diseases*. (J. Mawman, London, 1815).
137. W. Lambe, "Reports on the effects of a peculiar regimen on scirrhous tumors and cancerous ulcers," (London, 1809).
138. T. C. Campbell, Nutrition and cancer: an historical perspective--the past, present, and future of nutrition and cancer. Part 2. Misunderstnading and ignoring nutrition. *Nutritional Cancer*, 1-7 (2017).
139. T. C. Campbell, The past, present, and future of nutrition and cancer: Part 1-Was a nutritional association acknowledged a century ago? *Nutrition and Cancer* **69**, 811-817 (2017).
140. C. Tomasetti, B. Vogelstein, Variation in cancer risk among tissues can be explained by the number of stem cell divisions. *Science* **347**, 78-81 (2015).
141. C. Wild *et al.*, Cancer risk: role of chance overstated. *Science* **347**, 728 (2015).
142. N. A. Ashford *et al.*, Cancer risk: role of environment. *Science* **347**, 727 (2015).
143. M. O'Callaghan, Cancer risk: accuracy of literature. *Science* **347**, 729 (2015).
144. J. D. Potter, R. L. Prentice, Cancer risk: tumors excluded. *Science* **347**, 727 (2015).
145. M. Song, E. L. Giovannucci, Cancer risk: many factors contribute. *Science* **347**, 728-729 (2015).
146. C. Gotay, T. Dummer, J. Spinelli, Cancer risk: prevention is crucial. *Science* **347**, 728 (2015).
147. J. Krier, R. Barfield, R. C. Green, P. Kraft, Reclassification of genetic-based risk predictions as GWAS data accumulate. *Genome Med* **8**, 20 (2016).
148. B. L. Brucher, I. S. Jamall, Somatic mutation theory - why it's wrong for most cancers. *Cell Physiol. Biochem.* **38**, 1663-1680 (2016).
149. P. J. Stephens *et al.*, The landscape of cancer genes and mutational processes in breast cancer. *Nature* **486**, 400-404 (2012).
150. M. L. Peters, J. F. Tseng, R. A. Miksad, Genetic Testing in Pancreatic Ductal Adenocarcinoma: Implications for Prevention and Treatment. *Clinical therapeutics* **38**, 1622-1635 (2016).
151. A. B. Kroigard, M. J. Larsen, M. Thomassen, T. A. Kruse, Molecular Concordance Between Primary Breast Cancer and Matched Metastases. *Breast J*, (2016).
152. L. M. Randall, B. Pothuri, The genetic prediction of risk for gynecologic cancers. *Gynecol Oncol* **141**, 10-16 (2016).
153. M. El Zoghbi, L. C. Cummings, New era of colorectal cancer screening. *World J Gastrointest Endosc* **8**, 252-258 (2016).
154. E. K. Weisburger, Mechanisms of chemical carcinogenesis. *Ann. Rev. Pharmacol. Toxicol.* **18**, 395-415 (1978).
155. F. H. Cafferty, R. E. Langley, Polypill is not just for cardiovascular disease. *BMJ* **357**, j2733 (2017).
156. G. P. Pfeifer *et al.*, Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. *Oncogene* **21**, 7435-7451 (2002).
157. R. B. Shekelle, W. J. Raynor Jr., Dietary vitamin A and risk of cancer in the Western Electric Study. *Lancet* **2**, 1185-1190 (1981).
158. D. P. Rose, A. P. Boyar, E. L. Wynder, International comparisons of mortality rates for cancer of the breast, ovary, prostate, and colon, and per capita food consumption. *Cancer* **58**, 2363-2371 (1986).
159. V. Aran, A. P. Victorino, L. C. Thuler, C. G. Ferreira, Colorectal Cancer: Epidemiology, Disease Mechanisms and Interventions to Reduce Onset and Mortality. *Clin Colorectal Cancer*, (2016).
160. S. Nik-Zainal *et al.*, The genome as a record of environmental exposure. *Mutagenesis* **30**, 763-770 (2015).
161. J. Kaiser, When less is more. *Science* **355**, 1144-1146 (2017).
162. R. Elias, E. Karantanos, K. L. Sira, L. Hartshorn, Immunotherapy comes of age: immune aging & checkpoint inhibitors. *J. Geriatr. Oncol.* **3**, 229-235 (2017).
163. L. M. Schuman, The benefits of cessation of smoking. *Chest* **59**, 421-427 (1971).
164. T. P. O'Connor, B. D. Roebuck, T. C. Campbell, Dietary intervention during the post-dosing phase of L-azaserine-induced preneoplastic lesions. *J Natl Cancer Inst* **75**, 955-957 (1985).
165. G. J. Mulder, *The Chemistry of vegetable & animal physiology (translated by P.F.H. Fromberg)*. (W. Blackwood & Sons, Edinburgh, London, 1849), pp. 827.
166. G. J. Mulder, *(paper where he named Protein, according to Munro, 1964)*. *J. Prakt. Chem.* **16**, 29 (1839).
167. J. W. Lampe *et al.*, Plasma isoflavones and fibrocystic breast conditions and breast cancer among women in Shanghai, China. *Cancer Epidemiol Biomarkers Prev* **16**, 2579-2586 (2007).
168. N. Kurahashi *et al.*, Soy product and isoflavone consumption in relation to prostate cancer in Japanese men. *Cancer Epidemiol Biomarkers Prev* **16**, 538-545 (2007).
169. S. Banerjee, Y. Li, Z. Wang, F. H. Sarkar, Multi-therapy of cancer by genistein. *Cancer Lett.* **269**, 226-242 (2008).
170. H. Si, D. Liu, Phytochemical genistein in the regulation of vascular function: new insights. *Curr. Med. Chem.* **14**, 2581-2589 (2007).
171. H. Marini *et al.*, Effects of the phystoestrogen genistein on bone metabolism in osteopenic postmenopausal women: a randomized trial. *Ann. Int. Med.* **146**, 839-847 (2007).
172. E. Giovannucci, Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. *J Natl Cancer Inst* **91**, 317-331 (1999).
173. D. Ilic, Lycopene for the prevention and treatment of prostate disease. *Recent Results Cancer Res* **202**, 109-114 (2014).
174. X. Qiu *et al.*, Effects of lycopene on protein expression in human primary prostatic epithelial cells. *Cancer Prev Res (Phila)* **6**, 419-427 (2013).
175. S. Reuter, S. C. Gupta, M. M. Chaturvedi, B. B. Aggarwal, Oxidative stress, inflammation, and cancer: how are they linked? *Free Radic Biol Med* **49**, 1603-1616 (2010).
176. D. Ornish *et al.*, Can lifestyle changes reverse coronary heart disease? *Lancet* **336**, 129-133 (1990).
177. C. B. Esselstyn, Jr., Updating a 12-year experience with arrest and reversal therapy for coronary heart disease (an overdue requiem for palliative cardiology). *Am. J. Cardiol.* **84**, 339-341 (1999).
178. C. Esselstyn, M. Golubic, The nutritional reversal of cardiovascular disease, Fact or Fiction? Three case reports. *Exper. Clin. Cardiol.* **20**, 1901-1908 (2014).
179. G. K. Davey *et al.*, EPIC-Oxford: lifestyle characteristics and nutrient intakes in a cohort of 33 883 meat-eaters and 31 546 non meat-eaters in the UK. *Public Health Nutr* **6**, 259-269 (2003).
180. J. G. Sobiecki, P. N. Appleby, K. E. Bradbury, T. J. Key, High compliance with dietary recommendations in a cohort of meat eaters, fish eaters, vegetarians, and vegans: results from the European Prospective Investigation into Cancer and Nutrition-Oxford study. *Nutr Res* **36**, 464-477 (2016).
181. N. Pritikin, P. M. McGrady, *The Pritikin Program for Diet and Exercise*. (Bantam Books, 1984).
182. J. A. McDougall, M. A. McDougall, *The McDougall Plan*. (New Win Publishing, Inc., Clinton, NJ, 1983), pp. 340.
183. L. M. Morrison, Diet in coronary atherosclerosis. *JAMA* **173**, 884-888 (1960).
184. C. B. J. Esselstyn, G. Gendy, J. Doyle, M. Golubic, M. F. Roizen, A way to reverse CAD? *J Fam. Pract.* **63**, 356-364b (2014).

185. D. Ornish *et al.*, Intensive lifestyle changes for reversal of coronary heart disease. *JAMA* **280**, 2001-2007 (1998).
186. D. Ornish, Avoiding revascularization with lifestyle changes: the Multicenter Lifestyle Demonstration Project. *Am. J. Cardiol.* **82**, 72T-76T (1998).
187. M. Siddiqui, S. V. Rajkumar, The high cost of cancer drugs and what we can do about it. *Mayo Clinic proceedings* **87**, 935-943 (2012).
188. C. Hunter *et al.*, A hypermutation phenotype and somatic MSH6 mutations in recurrent human malignant gliomas after alkylator chemotherapy. *Cancer Res* **66**, 3987-3991 (2006).
189. L. Szabo, in *USAToday*. (Online, 2017).