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Review

Context is everything: Mining the normal and preneoplastic microenvironment for insights into the diet and cancer risk conundrum

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This review highlights the context-dependence of epithelial carcinogenesis in order to illuminate the potential for progress in the field of diet and cancer prevention. Estimates drawn from observational epidemiology imply that diet and lifestyle changes have the potential to prevent 30–40% of cancer cases. However, the application of knowledge gleaned from observational epidemiology applied to randomized clinical trials (RCT) has yielded equivocal or negative results. Resolving this conundrum requires: (i) advances in diet assessment methodologies and the design of clinical trials; (ii) greater knowledge of the active components within foods which may impact cancer risk; and (iii) knowledge about the effects of dietary components on susceptible tissues throughout the disease process (Meyskens, F. L., Jr., Szabo, E., Diet and cancer: The disconnect between epidemiology and RCT. *Cancer Epidemiol. Biomarkers Prev.* 2005, *14*, 1366–1369). Explicit consideration of the causal criteria will pay tangible benefits in the design of basic, clinical, and epidemiologic studies in cancer prevention. The rational identification of diet-dependent physiologic targets for cancer prevention is best pursued by appreciating context-dependence of epithelial carcinogenesis. Five contexts, or paradigms useful in understanding the multifactorial nature of carcinogenesis, are offered which describe the potential diet-associated physiologic influences on normal and preneoplastic cells and tumor microenvironments. Taken together with the interactions of systemic, endocrine, and autocrine/paracrine signals that may modulate the process of carcinogenesis, we can appreciate how dietary factors may act collectively in normal tissues or at early stages of carcinogenesis to prevent cancer. Only by understanding the effect of dietary components on the cellular and stromal components of the tissue microenvironment early in the process of epithelial carcinogenesis will yield clues useful for the development of improved strategies for cancer prevention.

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1 Diet and cancer prevention: Conundrums provide learning opportunities

Significant progress has been made in understanding the role of diet in cancer risk and prevention. Estimates drawn largely from observational epidemiology state that diet has the potential to prevent 30–40% of cancer cases [1]. This progress is evidenced by the existence of remarkably consistent dietary recommendations to prevent cancer from diverse public and private agencies. The American Cancer Society, the American Heart Association, and the American Diabetes Association released coordinate dietary recommendations for the prevention of cancer, coronary heart disease and to decrease comorbidities attributable to diabetes [2]. The fact that similar etiologic processes, such as inflammation, underlying these chronic diseases suggests that a single set of dietary recommendation may modulate at least some of the pathophysiologic processes they share.

Despite progress in understanding the elements of genetic and environmental susceptibility that interact to predict cancer risk, a significant conundrum has arisen from the application of knowledge gleaned from observational epidemiology to randomized clinical trials (RCT) to prevent cancer [3]. This conundrum – that RCTs testing the

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Abbreviations: CRC, colorectal cancer; **RCT,** randomized clinical trials

preventive efficacy of single nutrients (*e.g.*, β-carotene or retinol to prevent lung cancer [4]) or isolated food components (*e.g.*, wheat bran fiber in individuals with colon polyps [5]) in high risk populations yielded negative or null results – has produced confusion and skepticism about the role of diet in modulating cancer risk among the public, clinicians, funding agencies, and public policy makers.

Why is it that observational studies consistently show decreased cancer risk due to fruit and vegetable consumption [6] while clinical trials of foods [7], isolated nutrients from these foods or food components do not [5, 8]? The conundrum arises when results from observational and intervention epidemiologic trials performed in low risk populations are applied, without full consideration of the principles of causal inference (discussed below), to high risk populations. Epidemiologic studies show a strong inverse relationship between dietary fiber intake and colon cancer in populations at low risk for the disease [9, 10]. In contrast, secondary polyp prevention trials using wheat fiber or high-fiber, high-fruit-and-vegetable, low-fat dietary intervention have yielded null results [7, 11]. The confusing nature of these findings reveals disconnection between knowledge gleaned from observational epidemiology and the results of recent chemoprevention trials.

While the results of these trials are perplexing, they provide many learning opportunities. Meyskens and Szabo outlined important areas where knowledge is needed for advancements to occur in the field of cancer chemoprevention. The first of the three primary shortcomings they identified is the inability to identify nutrients/non-nutrients in the food (the "biological action package") that are associated with cancer preventive effects [3]. Second, the identification of all key regulatory components of the "biological action package" is needed to allow for a more precise epidemiological estimation of risk. Third, the failure to characterize the effect of the disease process itself on the effect of the biological action package is a severe limitation in the epidemiology of chemoprevention. These limitations have hindered our ability to translate findings from observational epidemiology into progress in cancer prevention.

These targeted areas for knowledge development described by Meyskens and Szabo provide a rational platform from which to assess the different contexts or stages of disease. Cancer is characterized by a decades-long process of transformation of a normal cell to a tumor cell. Due to current limitations in the assessment of preclinical neoplasia, the stage of this dynamic process is not assessed or defined in most RCTs. Clearly, large observational cohort studies utilizing genomic and proteomic approaches to associate dietary (and other environmental exposures) and genetic predispositions to cancer risk have great promise to clarify this conundrum [12]. However, full consideration of the rules of evidence will be necessary in order to glean useful information from such studies [3]. The next section will offer evidence from philosophical and biological perspectives that may allow for the development of approaches to diet and cancer prevention that account for the context-dependence of epithelial carcinogenesis.

2 Causal inference: A necessary preassessment tool for scientists in cancer prevention

An opportunity exists to apply the principles of causal inference to understand the results of recent chemoprevention trails. It is axiomatic that a certain level of evidence must be available to scientists planning a chemoprevention study before the decision to invest significant resources in prevention trials. Causal inference provides a paradigm in which epidemiologic evidence can be assessed to determine whether a relationship exists between an exposure and an outcome [13]. In epidemiology, the assessment of the strength of the relationship between exposure and outcome requires consideration of widely used causal criteria [14]. These criteria include, the establishment of strong exposure-outcome relationships from data derived from prevention trials. Further, strict application of these principles requires, (i) consistent and statistically significant results across many different studies, (ii) large relative risk estimates, (iii) extensive understanding of biological mechanisms and dose-response relationships, (iv) positive prevention trial results, (v) a clear temporal relationship between exposure and outcome, and other conditions [14, 15]. These criteria are inclusive of the identified knowledge needs set forth by Meyskens and Szabo and provide additional criteria which epidemiologists should consider. It is critical that future studies apply these principles to the existing data from epidemiological studies, and perhaps data from the clinical and basic sciences, to inform the design of prevention trials.

Since the intervention trials cited above did not mention the explicit use of causal criteria in their design, we can impute that the causal criteria of biologic plausibility and dose-response played a large role in the justification of these trials while the application of other principles is less clear. The rigorous application of the principles of causal inference requires consideration of additional causal criteria besides biological plausibility and dose-response prior to investments into randomized, controlled clinical chemoprevention trials. Advancing knowledge of the biology of carcinogenesis suggests it would be beneficial to include other critical information in the assessment of epidemiologic data for the optimal design of chemoprevention trials.

3 Defining the contexts of carcinogenesis

Cancer is known to result from the interaction of environmental and genetic susceptibilities. Therefore, it is critical

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that we identify, in a tissue-specific manner, the factors affecting the neoplastic transformation of normal tissue [16]. This knowledge includes identifying the transformational event, perhaps an initiating mutation in stem cell, stem cell progenitor or cancer stem cell. The decades-long process of clonal expansion enables these initiated cells to develop into a tumor. The process of clonal expansion produces morphologic changes represented as "stages" of tumor development. Each of these stages represents an opportunity for prevention of the next step of progression. Identifying the effects of dietary factors on normal tissues and susceptible tissues at each stage of neoplastic development is an important prevention strategy in need of significant research investment. These needs are clearly identified by the National Cancer Institute Think Tanks in Cancer Biology, nine groups of 15–25 experts in cancer biologist, immunologists, and clinicians brought together to identify emerging concepts in carcinogenesis and promising opportunities for investigation in cancer biology [16]: "Growth and migration of normal epithelial cells are subject to many levels of regulation by neighboring cells, extracellular matrix, and local levels of soluble signaling molecules. Cancer cells lose critical aspects of these controls, but they lose them gradually and rarely lose them all. Thus, one way of looking at cancer initiation and progression is an iterative and progressive renegotiation of constraints carried out between the developing clone of epithelial cancer cells and its stromal microenvironment."

Six of nine Think Tank groups highlighted the necessity to understand carcinogenesis in this context of normal biology as opposed to our historical focus on tumor biology. This recommendation to focus on early events in epithelial carcinogenesis should be a clarion call to scientists interested in cancer prevention [15]. The development of rational and effective dietary strategies of cancer prevention is sorely needed in light of the results of recent chemoprevention trials. This review will highlight the potential for progress in cancer prevention by providing insights into the role of dietary factors in the developing neoplastic microenvironment.

Understanding the effect of biological activity of foods or food components on disease processes presumes a knowledge of disease etiology and a thorough knowledge of the potential biological activities of food components. By taking a step back and considering recent developments in carcinogenesis, we may appreciate how dietary factors may collectively, and at earlier stages of carcinogenesis, prevent cancer. This section will present five contexts (defined as paradigms useful in understanding the multifactorial nature of carcinogenesis) which inform about the process of cancer prevention research. Reflection on these contexts may assist in identifying gaps in existing knowledge by identifying heretofore unacknowledged or underappreciated cellular targets for dietary compounds and relevant collaborations among cell types in the developing neoplastic microenvironment.

Each context describes factors which influence the microenvironment of the epithelial cell, including dietary, physiologic, endocrine, and autocrine/paracrine factors as well as the dynamic, interactive nature of heterogeneous cell types, and extracellular matrix components. Each context is not reviewed exhaustively. However, since each context suggests multiple effect modifiers, these should be accounted for, if possible, in the design and analysis of studies in diet and cancer prevention. Each context may also reveal biomarkers of stage or biomarkers which are amenable to dietary factors which have been unidentified or underappreciated.

Mining the normal epithelial microenvironment and developing neoplastic microenvironment for clues to advance progress in cancer prevention research requires identifying the cell types that participate in neoplastic development, characterization of the types of necessary interactions between stem cells and cell co-opted into neoplastic process, and identification of the stage-specific biomarkers which develop over time that are associated with progression.

4 The contexts of epithelial carcinogenesis: Identifying the players

4.1 Context 1: The physiologic context of energy balance

If we were to rank the afferent signals from the diet which most potently affect carcinogenesis, caloric restriction would be first. The most effective dietary intervention to prevent cancer and delay aging in experimental animal models is caloric restriction [17]. Restriction of caloric intake to 60–80% of *ad libitum* calories prevents sporadic and carcinogen-induced cancer by approximately 20–40% depending upon the animal model [17]. Since adjustment for energy intake is a standard statistical technique employed in nutritional and cancer epidemiology studies, mention of this context is brief but necessary.

The mechanistic determinants of the effects of caloric restriction are not known but may relate to the relative dearth of anabolic signals to susceptible tissues from hypoinsulinemia and low levels of the insulin-like growth factors [18]. It is tempting to implicate a role for caloric restriction in the maintenance of adult stem cells or stem cell progenitors in G_0 phase of the cell cycle, the basal state of adult stem cells [19], but no data exist to evaluate these presumptions.

The strongest association between diet-related factors and cancer risk is overweight and obesity [20]. The World Health Organization estimated in 2002 that approximately 8–42% of certain cancers globally were attributable to a BMI (weight (kg)/height (m^2)) above 21 kg/m^2 [21]. At the physiological level, the effects of overweight and obesity on

cancer risk has been attributed to elevated levels of hormones, cytokines, and other biologically active factors secreted by adipocytes [22].

As an important set of diet-related physiologic signals affecting carcinogenesis, adipocytokines provide efferent signals to the neoplastic microenvironment typically originating from abdominal adipose tissue in overweight or obese individuals. The mechanistic underpinnings of obesity-associated cancers are poorly understood. Recent evidence from *in vitro* models of normal and preneoplastic colon epithelial cells suggest that the obesity-associated, adipocyte-derived hormone, leptin, may preferentially cause proliferation of preneoplastic, but not normal, epithelial cells [23, 24]. These data highlight the necessity to assess the effect of neoplastic phenotype-associated biological factors, like leptin, early in the process of neoplastic development.

4.2 Context 2: The context of the neoplastic potential of tissue type

Carcinoma refers to a malignant neoplasm of epithelial origin or cancer of the internal or external lining of the body. Carcinomas account for 80–90% of all cancer cases [25]. The fact that most adult cancers occur in epithelial microenvironments suggest that these tissues uniquely supply access to susceptible cells by dietary compounds, immune cells, stromal cells, and tissue components that can influence initiation and subsequent neoplastic transformation.

The specific cell type responsible for transformation in epithelial carcinogenesis is not known. Epithelial cell biology, including the biology of self-renewing stem cells that serve these tissues, is the stage on which to view the chronic process that results in autonomous neoplastic growth and metastasis. Accumulating evidence suggests that adult tissues contain stem cells or stem cell progeny (referred to hereafter as progenitors) which are the targets of transformational events that drive tumorigenesis [19]. Whether the cells which drive tumorigenesis are stem cell, cancer stem cell, stem cell progenitor, a dedifferentiated epithelial cell or an associated stromal cell is beyond the scope of this review. Given that the preponderance of the data support a role for cancer stem cells or their progenitors in tumorigenesis [26], this review cites these cell types as the most likely culprits.

Far from being solitary player in carcinogenesis, the stem cell or its progenitors that acquire transformational mutations may be influenced by the heterogeneous cell types and extracellular matrix components in its microenvironment. Stromal cells (*e.g.*, endothelial cells, fibroblasts, myoepithelial cells, inflammatory cells, *etc.*) surrounding the neoplastic epithelial cells are now thought to play critical roles in influencing, and in some cases inducing, the behavior of the tumor [27, 28]. Stromal cell activation could result from signals from surrounding cells that undergo transient/stable epigenetic changes, random or occasionally targeted/evolutionarily selectable genetic changes, and/or from the recruitment of appropriate cells (*e.g.*, inflammatory cells) to produce secreted products or other factors that regulate all aspects of tumorigenesis [29, 30]. The identification of stage-specific markers from the early phases of neoplastic development, perhaps first in *in vitro* models of cellular transformation, would serve progress in the field of cancer prevention.

4.3 Context 3: The context of stage-phenotypes associated with carcinogenesis

An important consideration, allied conceptually with the causal criteria of biologic plausibility and dose-response, is the effect of disease stage on the biological activity of dietary factors [3]. This subject was recently reviewed in the context of utilizing appropriate *in vitro* models in cancer prevention research [15]. Full consideration of effect of cancer stage and other potential effect modifiers on carcinogenesis is not possible due to our incomplete knowledge of carcinogenesis (including the role of stem cell in the genesis of cancer) and the dearth of tissue-specific or plasma biomarkers of early- and intermediate-term events in cancer risk. However, the assumption implicit in the design of chemoprevention trials – that dietary factors associated with decreased cancer risk from epidemiologic observations can be applied individually and in pharmacologic doses to produce similar effects – has been demonstrated to be, for the most part, simplistic if not harmful.

Hanahan and Weinberg [31] offer support for the hypothesis that the process of carcinogenesis is the result of the acquisition of six phenotypes characteristic of neoplastic development resulting in cellular immortality. It has been persuasively argued that stem cell oncogenesis and decreased cell–cell communication between stem cells/stem cell progenitors and normal progenitor cells should be added to this list of required phenotypes for carcinogenesis [31]. While each of these phenotypes are well-characterized, the temporality and combination of phenotypes which enable neoplastic growth for each tissue is not known. The acquisition of these phenotypes, viewed morphologically as stages of disease progression, presents a temporal dimension wherein each acquired phenotype supports enhanced cell survival and the opportunity for acquisition of additional neoplasia-associated phenotypes over the decades-long course of carcinogenesis. The temporality of neoplastic development can be appreciated by applying these phenotypes to a hypothetical sequence during colorectal carcinogenesis. In colorectal cancer (CRC), the acquisition of mutations in the *adenomatous polyposis coli* gene leads to activation of the Wnt/β-catenin/Tcf-LEF pathway; the initiating event in familial adenomatous polyposis and in most sporadic CRC cases [32]. This simultaneously leads to the acquisition of two cancer-associated phenotypes: evasion of apoptosis and sustained angiogenesis through ß-catenin/

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Tcf-LEF-mediated c-myc, and cyclo-oxygenase-2 production. Insensitivity to antigrowth signals is acquired with mutations in the Smad proteins; the downstream effectors of the differentiation-promoting transforming growth factor-β (TGF-β) produced by epithelial cells and T regulatory cells [33]. Self-sufficiency in growth signals is acquired with oncogenic activation of the Ras-Raf-MAPK signaling cascade, seen in over 50% of CRC cases [34]. Limitless replicative potential is the hallmark phenotype indicative of telomerase activation [35].

4.4 Context 4: The context of innate and acquired immune cell participation in carcinogenesis

The growing literature associating the activation of innate and adaptive immune cells in carcinogenesis is difficult to summarize briefly. The role of the inflammation in cancer initiation and progression can be characterized as "a wound that does not heal". The inflammatory response is characterized by an innate immune response, initiated by local or systemic insults [36]. This typically results in an acute response followed by a late phase where tissue repair and remodeling resolves the infection and its effects. In cancer, however, the late phase does not resolve; the resulting chronic local inflammatory reactions, with little systemic manifestations, provide the background for the pathophysiology of most neoplastic conditions [36].

Mechanistic support for these associations between chronic inflammatory processes and cancer has been provided by experiments using elegant transgenic animal models or adoptive transfer of specific immune cell types into susceptible hosts. These studies identified specific roles for innate or adaptive immune cells, their secreted products, or stromal cells in tumorigenesis [37, 38]. The conversations which develop the putative stem cell population and surrounding cell types, including stromal, epithelial, immune, fibroblasts, and endothelial cells, involve the release of solid (*e.g.*, extracellular matrix protein structures) and soluble (*e.g.*, growth factors, chemokines, cytokines, nitric oxide, *etc.*) factors which can facilitate tumor progression or prevention of tumor growth [38].

Emerging hypotheses concerning the genesis of *Helicobacter pylori*-induced gastric cancer in a murine model provides an interesting example of how stem cells and inflammatory stimuli interact to impact cancer risk. These data have led to the surprising insight that gastric oncogenesis in this model may originate from circulating bone marrow-derived stem cells (BMDC) and not from resident tissue stem cells as previously believed [39, 40]. This raises the prospect that epithelial carcinogenesis could be driven by stem cells from bone marrow or other distal tissues. This model of gastric cancer corroborates findings in human patients, in whom disease outcome is largely determined by the expression of host proinflammatory cytokines [39]. It is likely that this new BMDC paradigm of epithelial cancer will prove useful in future investigations of cancers associated with chronic inflammation.

These data show that a delicate balance between collaborating cell types in the epithelial stem cell microenvironment are critical determinants of the development of the phenotypes associated with neoplastic development. Dietary factors which prevent cancer may be acting by interdicting the conversations among cells in the early neoplastic microenvironment; state-of-the-art imaging techniques may be able to provide the resolution to witness these preventive events *in vivo*.

4.5 Context 5: The concentration of diet-derived compounds in the neoplastic microenvironment

The effects of nutrient and non-nutrient components and their bioavailability within foods present a complex mixture of food components, nutrients, and their metabolites at target tissues after food consumption. It can be assumed that pharmacological doses of nutrients have disparate biological effects compared to the effects of the same nutrient at physiological concentrations resulting from food consumption. A chemoprevention trial designed to test the hypothesis that pharmacologic doses of alpha-tocopherol, β-carotene, or both supplements taken together would decrease lung cancer risk in male long-term smokers proved this assumption true [4]. However, the carcinogenic effects of β-carotene supplementation is contrasted by the predictive value of baseline serum β-carotene concentrations on lung cancer risk in this cohort [8]. These data suggest that pharmacologic levels of β-carotene may have exerted carcinogenic effects and that baseline serum β-carotene was a surrogate marker for unidentified protective components in the diet. Alternatively, baseline serum β-carotene levels may have reflected long-term exposure and that this type of exposure resulting from dietary β-carotene sources that contained compounds which were protective against the initiation phase of lung carcinogenesis.

Folic acid consumption provides an additional illustration of the principle that the timing and form of the exposure of a dietary compound are important considerations in their effects on disease susceptibility. The relationship between folate and CRC carcinogenesis is complex, while low-folate status early in life may enhance CRC carcinogenesis; excess folate intake may be harmful in certain situations [41]. In animal models, folate supplementation is an effective chemopreventive agent if given prior to the establishment of early colorectal lesions. However, once a preneoplastic lesion is present, folate has been shown to enhance tumor growth [42]. Further evidence from a randomized controlled trial demonstrated that folic acid supplementation among patients with resected adenoma enhanced the recurrence of multiple or larger adenomas [43]. These findings highlight possible deleterious effects of high folic

acid intakes. The question is raised by Ulrich and Potter [44], relevant to any dietary factor with life stage- or disease stage-specific effects, is "Do the deleterious effects of high folic acid intakes outweigh the known and potential benefits?". In the case of folic acid, due to the high prevalence of folic acid in dietary supplements and fortified foods, it is an urgent question to be addressed.

The complexity of relating dietary exposures to cancer risk includes issues of related to developmental stage (*e.g.*, life stage), duration (*e.g.*, acute *vs*. chronic consumption), how much (*e.g.*, plasma concentration) and chemical form (*e.g.*, purified *vs*. food matrix) food components affect specific tissues. As emphasized above, all may be important and may determine how dietary factors affect cancer risk in the epithelial microenvironment.

Since specific dietary factors can modulate cancer risk, it is reasonable to assume that these effects are mediated through direct influence on stem cell populations or collaborating cell types. Just as the biological activities of dietary compounds are different depending on the microenvironment, or stage, similarly, their biological impacts are likely to be seldom singular [45]. In fact, animal and *in vitro* models have been used to demonstrate the efficacy of dietary compounds to affect multiple biological activities associated with the cancer-associated phenotypes discussed above. Illustrative examples of dietary compounds that act at multiple stages of carcinogenesis to prevent cancer include curcumin [46] and resveratrol [47]. The multifunctional nature of curcumin and resveratrol in preventing carcinogenesis contrasts with the aforementioned hypothetical preventive effect of folic acid at the preinitiation, but not postinitiation, phase of carcinogenesis. Thus, it is critical that chemopreventive agents be tested in relevant *in vitro* and animal models prior to the design of human trials. The identification of tissue- and stage-specific biomarkers of these phenotypes could be useful in predicting the outcome of chemopreventive agents on cancer risk.

5 Summary

The potential for the prevention of cancer by dietary means has inspired secondary chemoprevention trials that yielded null or negative results. Inadequate assessment of evidence from epidemiologic and basic science studies may have led to the emphasis of the causal criteria of biological plausibility and dose-response to the exclusion of other criteria. These aforementioned chemoprevention trials reveal a simplistic understanding of cancer biology and the preventive efficacy of nutrients and food components that result in either no preventive effect or an increase in risk. Advances in understanding the role of the dietary components on normal versus the developing neoplastic microenvironment will help identify important targets which may be amenable to dietary modulation. Critical epithelial–stromal reciprocal signaling pathways as therapeutic targets during the earliest stages of premalignant development should be identified [30]. Understanding the dependence of the preventive efficacy of dietary components on temporality (*e.g.*, stage), concentration in the normal or neoplastic microenvironment, and cellular and stromal components of the normal and neoplastic microenvironments holds the key for progress in cancer prevention research. Insights from appropriate nontumorigenic *in vitro* models and emerging paradigms in carcinogenesis may help avoid the undertaking of chemoprevention trials which yield more harm than good. Providing multiple dietary factors to normal or early stages of neoplastic development has the greatest promise to decrease risk of cancer.

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