The ultimate goal of carcinogenesis research is to achieve an understanding of the processes involved in the induction of human cancer to an extent that will allow intervention to prevent the disease, either in the general population or in susceptible sub-populations. Humans are exposed to a wide variety of carcinogenic insults, including chemicals, viruses, radiation, and physical agents [1]. In addition, it has become increasingly apparent that an individual’s genetic background can dramatically affect the level of susceptibility to carcinogenic exposure [2–4]. The objective of this chapter is to examine the basic mechanisms and stages of the carcinogenesis process, with an emphasis on the ways in which food components can modify those mechanisms.

Overview of Multistage Carcinogenesis

The major stages of carcinogenesis, deduced primarily from animal model studies over the past 50 years, are termed initiation, promotion and progression [5] and are depicted in Figure 1. Tumor initiation begins in cells with DNA damage resulting from exposure of that cell’s genetic material to exogenous or endogenous carcinogens. Unless repaired, DNA damage results in genetic mutations, which can alter the responsiveness of the mutated cells to their microenvironment, eventually providing them with a growth advantage relative to normal cells. However, initiation itself is not sufficient for tumor development. For example,
Nutrition and Carcinogenesis

Fig. 1. Targets for the nutritional modulation of the carcinogenesis process. Schematic presentation of the stages in experimental carcinogenesis and prevention strategies. The initiation stage is depicted as the conversion of a normal cell to an initiated cell in response to DNA-damaging agents, with the genetic damage indicated by an X. The promotion stage is depicted as the transformation of an initiated cell into a preneoplastic cell due to alterations in gene expression and cell proliferation. The progression stage is shown as the transformation of a preneoplastic cell to a neoplastic cell as a result of additional genetic alterations (indicated by additional Xs). Stage-specific strategies for dietary prevention are depicted as brick walls along the pathway.

in the classic 2-stage carcinogenesis system in the mouse skin, a low dose of 7,12-dimethylbenzanthracene (DMBA) will result in permanent DNA damage but will not give rise to tumors over the lifespan of the mouse unless a tumor promoter, such as 12-O-tetradecanoylphorbol 13-acetate (TPA) is repeatedly applied [5]. The tumor promotion stage is characterized by clonal expansion of initiated cells due to alterations in the expression of genes whose products are associated with hyperproliferation, decreased apoptosis, tissue remodeling and inflammation [6]. During the tumor progression stage, preneoplastic cells develop into invasive tumors through further clonal expansion, usually associated with altered gene expression and additional genetic damage due to progressive genomic instability [7].

Given the multistage nature of carcinogenesis and our advancing knowledge of the critical processes involved at each stage, strategies for the nutritional prevention of cancer must focus on stopping the carcinogenesis process at the earliest
possible point in the pathway through mechanism-based approaches. As shown in Figure 1, potential targets for dietary modulation of the initiation stage include: (1) modifying carcinogen activation by inhibiting the enzymes responsible for that activation; (2) enhancing carcinogen detoxification by altering the level or activity of detoxifying enzymes; (3) improving direct scavenging of DNA-reactive electrophiles, and (4) enhancing DNA repair processes. Also depicted in Figure 1 are targets for blocking the processes involved in the promotion/progression stages of carcinogenesis, including: (1) scavenging reactive oxygen species (ROS), which in addition to acting at the initiation stage to damage DNA can also act at later stages to alter cell signaling processes; (2) altering the expression of cancer-related genes, such as oncogenes and tumor suppressor genes; (3) decreasing inflammation; (4) suppressing proliferation, and (5) encouraging apoptosis. In the following sections, each of these potential targets for interfering with the carcinogenesis process will be examined in greater detail, and examples of their modulation by nutritional factors (frequently in the mouse multistage skin carcinogenesis model, where stage-specific events have been best studied) will be discussed.

**Anti-Initiation Strategies: Targets and Examples**

**Carcinogen Activation**

Most chemicals are not carcinogenic, but a wide variety of chemicals can cause cancer in humans and animals [1]. Most chemical carcinogens cause DNA damage by reacting with DNA bases and are thus termed genotoxic. The carcinogens form covalent adducts with DNA in the nucleus and mitochondria. Endogenous carcinogens – which are often ROS generated as part of normal oxidative metabolism or as a result of the metabolism of xenobiotic compounds, as well as by ultraviolet radiation and gamma radiation – can also cause extensive DNA damage.

Metabolic activation of procarcinogens (i.e., carcinogens requiring enzymatic conversion to DNA-reactive intermediates) is generally catalyzed by cytochrome P450 enzymes through oxidation. More than 100 distinct mammalian P450 enzymes have been identified [8]. In addition, there are other enzyme systems involved in carcinogen activation, such as peroxidases (including the cyclooxygenases, which will be discussed in more detail below) and certain transferases such as N-acetyltransferase and sulfotransferase [9, 10]. Each of these enzymes provides a potential target for modulating carcinogen activation.

Fruits, vegetables and other foodstuffs contain numerous chemical constituents known to interact with the metabolic activation of chemical carcinogens. Examples of food sources containing agents that modify carcinogen activation are the cruciferous vegetables, such as cauliflower, broccoli and cabbage. The crucifers are sources of isothiocyanates, which are known to modify the metabolism of nitrosamines. Several investigators [11–14] have shown conclusively that the
metabolism and carcinogenicity of the tobacco carcinogen 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone is modified by the administration of phenethylisothiocyanate. Extensive structure-activity studies by these investigators have also shown that changing the carbon chain length of the isothiocyanate moiety correlates with tumor prevention apparently by making the isothiocyanate more suitable for insertion into the cell due to increases in lipophilicity [15]. Also, diallyl sulfide, a common volatile in garlic, has been shown to be a potent inhibitor of cytochrome P450 2E1 [16]. This cytochrome P450 metabolizes ethanol, acetone and several known chemical carcinogens, including several nitrosamines, which target the nasal tissues, oral cavity, liver and esophagus, as well as dimethylhydrazine and its metabolites known to induce colon tumors in rodents [17].

Another class of compounds under study is the coumarins, which are widely distributed in nature and are found in all parts of plants [18]. Earlier studies had suggested that, as a general class, coumarins could modulate drug-metabolizing enzymes and cytochromes P450 [19]. Cai et al. [20] have recently shown that several naturally occurring coumarins possess the ability to block skin tumor initiation by polycyclic aromatic hydrocarbons such as benzo(a)pyrene and DMBA through inhibition of P450s involved in the metabolic activation of these carcinogens. Other agents that are known to exert effects at this stage are listed in Table 1.

**Carcinogen Detoxification**

A series of enzymes (referred to as phase II enzymes) are involved in the detoxification of activated carcinogens, thus preventing their binding to DNA. In particular, the induction of the glutathione S-transferases (GSTs) is an important response for the detoxification of xenobiotics [21]. This class of enzymes functions to couple a number of diverse substrates to glutathione, enhancing excretion of xenobiotics from the body. GSTs are segregated into three classes based on their sequence homology and specificity for substrates [22]. Other detoxification enzymes include uridine 5'-diphosphate (UDP)-glucuronosyltransferase, the epoxide hydrolases, and quinone reductase [10, 23]. The ultimate carcinogenicity of a particular xenobiotic is largely determined by the efficiency with which these and other enzymes detoxify carcinogens.

Detoxification of chemical carcinogens by enzymes such as GST and UDP-glucuronosyltransferase is enhanced by several constituents found in garlic and onion, cruciferous vegetables and certain spices. For example, induction of phase II enzymes in animals has been shown to follow oral exposure to diallyl sulfide and s-allyl cysteine found in garlic; both compounds enhance GST levels in the liver and colon [24]. As previously discussed, these organosulfur compounds are also known to inhibit the activity of several P450 cytochromes [16]. Thus, a dual effect of decreased carcinogen activation and enhanced carcinogen detoxification may underlie the tumor-inhibiting effects of diallyl sulfide and related compounds [25]. Similarly, isothiocyanates play a dual role in the suppression of carcinogen activation as well as enhancing carcinogen detoxification through in-
**Table 1. Examples of dietary factors that target specific stages of carcinogenesis**

<table>
<thead>
<tr>
<th>Prevention strategy</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tumor initiation</strong></td>
<td></td>
</tr>
<tr>
<td>Inhibit carcinogen activation</td>
<td>Epigallocatechin gallate (EGCG), selenium, phenyl-isothiocyanate (PEITC), indole-3-carbinol, coumarins, ellagic acid, genistein</td>
</tr>
<tr>
<td>Scavenge electrophiles</td>
<td>Ellagic acid, EGCG</td>
</tr>
<tr>
<td>Enhance carcinogen detoxification</td>
<td>Diallyl sulfide, PEITC, EGCG, N-acetylcysteine, resveratrol</td>
</tr>
<tr>
<td>Enhance DNA repair</td>
<td>Calorie restriction, EGCG, selenium</td>
</tr>
<tr>
<td><strong>Tumor promotion/progression</strong></td>
<td></td>
</tr>
<tr>
<td>Scavenge reactive oxygen species</td>
<td>Antioxidants (carotenoids, α-tocopherol, ascorbic acid, EGCG), selenium, calorie restriction</td>
</tr>
<tr>
<td>Alter cancer-related genes</td>
<td>Retinoids (vitamin A and its analogues), calorie restriction, monoterpenes (i.e., d-limonene)</td>
</tr>
<tr>
<td>Decrease inflammation</td>
<td>Calorie restriction, resveratrol</td>
</tr>
<tr>
<td>Suppress proliferation</td>
<td>Calorie restriction, selenium, genistein, retinoids</td>
</tr>
<tr>
<td>Induce differentiation</td>
<td>Retinoids, calcium, sodium butyrate</td>
</tr>
<tr>
<td>Encourage apoptosis</td>
<td>Retinoids, sodium butyrate, genistein, calorie restriction</td>
</tr>
</tbody>
</table>

Increased GST activity [12]. The phytoalexin resveratrol, found in grapes and other food products and a tumor inhibitor in several models, also has been shown to induce phase II enzymes such as quinone reductase [26]. Other dietary interventions that are known to exert effects at this stage are listed in Table 1.

**Electrophiles/Reactive Oxygen Species**

One common feature of the metabolic activation of all procarcinogens is that their ultimate DNA-reactive carcinogenic species are electrophilic [27]. Many direct-acting carcinogens also damage DNA through electrophilic intermediates [28]. Thus the electrophilicity of the ultimate carcinogenic species serves as a shared intervention target for many chemical carcinogens. The electrophilic metabolites may themselves be ROS and interact as such with DNA [29]. Oxygen free radicals may also be involved in a step required for activation of a procarcinogen, and thus the reactions involved in metabolic activation of carcinogens may release ROS which can in turn attack DNA. Thus, enhancing the direct scavenging of DNA-reactive intermediates constitutes a plausible strategy for modulating this early stage of carcinogenesis.
As reviewed by MacLeod and Slaga [27], several compounds have been proposed as scavengers for the ultimate electrophilic metabolites of carcinogens, such as benzo(a)pyrene diol epoxide (BPDE) in the case of benzo(a)pyrene. Earlier studies showed that several sulphydryl compounds, including cysteine and 2-mercaptoethanol, have been effectively used as nucleophilic traps for BPDE [30]. In addition, riboflavin has been shown to promote the detoxification of BPDE by enhancing hydrolysis [31]. Also, a group of plant phenols, notably ellagic acid, have been identified which react faciley with BPDE and thereby block the mutagenicity of BPDE in in vitro systems [32]. Ellagic acid has been shown to be an anticarcinogen in vivo, with protective activity against topically applied BPDE in the mouse skin model [33]. In addition, a major polyphenolic antioxidant found in green tea, epigallocatechin-3-gallate (EGCG), has been shown to have strong anticarcinogenic effects in several models, including the mouse skin, lung, forestomach, esophagus, duodenum, pancreas, liver, breast, and colon [34]. EGCG reportedly can trap activated metabolites of several procarcinogens [13].

**DNA Repair Processes**

The generation of DNA-reactive intermediates by most chemical carcinogens leads to the production of DNA adducts or other types of damage. As reviewed by Mitchell et al. [35], normal mammalian cells can efficiently remove DNA damage induced by carcinogens. Cells use different strategies to repair DNA damage, depending on the structure of the damage and its location in the genome. For example, small lesions (such as alkylated DNA bases) are repaired by a mechanism termed base excision repair [36]. This process involves removal of the damaged base followed by a “small cut-and-patch” repair involving removal of a few nucleotides. When methylation occurs at either the O\(^6\) or O\(^4\) positions of guanine or thymine, the modified bases can be repaired by the direct transfer of the methyl group to a methyltransferase [37]. Bulky carcinogen-induced DNA adducts and ultraviolet light photodimers can be repaired through a “large cut-and-patch” mechanism involving a region of approximately 27–29 nucleotides that includes the damaged bases; this is termed nucleotide excision repair [38]. The integrity of the genetic information is threatened not only by various environmental exposures but also by errors produced during normal DNA replication, for example, non-Watson-Crick base-pairing and slippage during DNA replication. To correct the errors resulting from such misreplication, cells have also developed a mismatch repair mechanism [36].

Although the gene products and the general mechanisms of DNA repair are understood fairly well in prokaryotes, knowledge of mammalian repair systems has only recently accumulated, and little is known about the influence of dietary factors on these processes. Calorie restriction [39, 40], EGCG [41], and selenium [42] have shown enhancing effects on unscheduled DNA synthesis and other measures related to repair capacity. In addition, folate deficiency has been associated with hypomethylation and decreased DNA repair capacity [43].
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Anti-Promotion/Progression Strategies: Targets and Examples

Scavenging Reactive Oxygen Species

ROS play an important role in a variety of normal processes within the body, including the immune response against pathogens, intracellular signaling, and vascular permeability. However, the accumulation of ROS as byproducts of normal energy metabolism, or in response to inflammatory conditions or ROS-generating environmental exposures (i.e., particulates in tobacco smoke), has been associated with the pathogenesis of cancer in rodents and man [29, 44]. Experimental studies have shown that ROS can act as both initiators and promoters of tumors by damaging critical cellular macromolecules such as DNA, proteins and lipids, and by acting as cell-signaling molecules, such as nitric oxide [29, 44]. Antioxidants, including ascorbic acid, α-tocopherol, several carotenoids, selenium, and several polyphenolic compounds found in green tea, spices and fruits and vegetables have been shown to effectively inhibit TPA promotion in the mouse skin [29]. High intake of these compounds has also been associated with decreased risk of a variety of cancers in epidemiologic studies [45]. In addition, calorie restriction, which is one of the best documented and most effective experimental manipulations for decreasing tumor development in rodents [46], including suppressing TPA-induced skin carcinogenesis, may exert its antitumor effects, in large part, by decreasing ROS production and enhancing antioxidant defenses. Calorie restriction decreases the rate of accumulation of oxidized DNA and protein that accompanies aging in rodents [47]. In addition, a number of intracellular antioxidant defense systems are reportedly enhanced by calorie restriction, including the levels of superoxide dismutase, catalase and glutathione peroxidase [48]. Thus, evidence is mounting that calorie restriction may decrease oxidative stress via decreased oxidant production and enhanced antioxidant capacity, although the mediating role of these effects in the retardation of tumorigenesis by calorie restriction has yet to be fully established. Other agents that are known to exert effects at this stage are listed in Table 1.

Alter the Expression of Genes Related to Cell Proliferation and Apoptosis

The tumor promotion phase of multistage carcinogenesis involves the clonal expansion of initiated cells. By definition and observation, tumor-promoting agents are not mutagenic like carcinogens but rather cause an alteration in the expression of genes whose products are associated with hyperproliferation, cell survival, tissue remodeling and inflammation [49]. At some point, the developing tumor constitutively expresses these genes and thus becomes tumor promoter-independent. The identification of the mechanisms by which tumor promoters elicit altered gene expression has been of great interest over the past decade, particularly because the characterization of critical events offers a target for the development of new prevention strategies. It has also become clear in the past few years that apoptosis and mitogenesis are equally important processes in maintain-
ing cell number homeostasis, and that the growth advantage manifested by initiated cells during promotion is usually the net effect of increased proliferation and decreased apoptosis. Thus, in addition to inflammation and cell proliferation, apoptosis has emerged as a critical target for prevention [50].

Changes in gene expression as a consequence of external tumor promoter stimuli usually result in the activation, or sometimes inactivation, of specific signal transduction pathways. The nature of the initial interaction of tumor promoters with the cell depends on the type of promoter. For example, TPA interacts with specific receptors, which are isoforms of protein kinase C (PKC) [51]. Other promoters, such as okadaic acid, are potent inhibitors of phosphatases, causing a net increase in the level of phosphorylated proteins, which is often an activating effect similar to the activation of kinases [49]. However, regardless of the disparity in initial signaling events, the key biologic and molecular changes they elicit, such as increased DNA synthesis, induction of ornithine decarboxylase, induction of growth factors and cytokines, and increased production of eicosanoids, are all similar. Furthermore, the elicited production and secretion of growth factors, cytokines and eicosanoids activate signaling pathways via receptors specific for these molecules. This overall alteration in signal transduction and gene expression results in the selection and growth of the initiated cell population.

The discovery of the interaction of phorbol esters with PKC represented a major breakthrough in understanding the action of TPA. The PKC pathway, an obligatory signal transduction pathway in cells, is normally activated by diacylglycerol, which is transiently formed during receptor-mediated turnover of phosphatidylinositol [51]. All tissues express at least one isoform of PKC, and generally the abundance of particular isoforms are tissue-specific, suggesting different functions for different isoforms. Although the function of the different isoforms in the epidermis is not known, PKC\(\eta\) is the most abundant and shows differentiation-associated expression [51]. It is normally not found in the basal or spinous layers but is highly expressed in the upper granular layer, suggesting that this isoform plays a critical role in the signaling pathway leading to differentiation. The identification of PKC as the major target for phorbol esters and other promoters, such as mezerein, indole alkaloids, and polyacetates, suggests that activation of PKC is a critical event in carcinogenesis [49]. By activating PKC, phorbol esters and related tumor promoters appear to bypass normal cellular mechanisms for regulating cell proliferation. Several oncogenes (particularly ras, as mentioned above), hormones, growth factors and cytokines are also known to activate this signaling pathway.

As discussed by Fischer and DiGiovanni [49], not all tumor promoters work through receptor-mediated mechanisms. Organic peroxides such as benzoyl peroxide, and hydroperoxides, such as t-butyl hydroperoxide, are examples. Also, unlike the phorbol esters, the peroxides require metabolic activation. Benzoyl peroxide undergoes a copper-dependent activation, resulting in the formation of the benzoyloxyl radical that can undergo \(\beta\)-scission to form phenyl radicals [52].
The molecular targets of benzoyl peroxide have not been elucidated, although it has been shown to produce macromolecular damage, particularly covalent adducts with proteins. Benzoyl peroxide is generally regarded as noncarcinogenic and nongenotoxic. However, it has been shown to induce single-strand breaks in DNA [53]. Thus, it is not clear if the mechanism of benzoyl peroxide as a tumor promoter is truly epigenetic in nature.

Although organic peroxides do not activate signal transduction pathways via a receptor-binding mechanism, they may modify signaling elements via alkylation or by altering the redox state of key proteins. Altered signaling by these mechanisms would require considerably higher doses than that elicited by receptor-dependent modulators, consistent with the observation that 20-mg doses of benzoyl peroxide are needed for tumor promotion, while only microgram doses of TPA are required [52].

Despite its importance, activation of PKC per se does not appear to be sufficient for mediating phorbol ester-induced hyperproliferation in vivo, in part because a major consequence of PKC activation in keratinocytes is the stimulation of terminal differentiation [54]. The regulation of keratinocyte proliferation and differentiation is a complex process and likely involves interaction of different cell types in the epidermis and dermis, as well as multiple signaling pathways within the keratinocytes themselves. Several receptor tyrosine kinases and their ligands appear to be linked to keratinocyte proliferation. The 4 major receptors are the: (1) epidermal growth factor receptor (EGFR); (2) insulin-like growth factor receptor; (3) basic fibroblast growth factor receptor, and (4) hepatocyte growth factor receptor [49]. All 4 of these receptors are expressed on the surface of keratinocytes; however, with the exception of transforming growth factor-α (the ligand for EGFR), the ligands for the receptors are produced by dermal fibroblasts or inflammatory cells and act in a paracrine manner.

Several lines of evidence suggest that tumor promoters generally enhance the expression of a number of growth factors and cytokines. TPA induces transforming growth factor (TGF)-α, TGF-β, tumor necrosis factor-α, granulocyte-macrophage stimulating factor, and interleukins (IL)-1 and 6 [49]. The profile of growth factor induction is different for promoters with differing initial mechanisms of action, although most seem to lead to induced levels of TGF-α mRNA expression [55]. Studies using transgenic mice which overexpress TGF-α in the basal cells and display epidermal hyperplasia and increased susceptibility to skin tumor promotion further support the hypothesis that TGF-α plays a critical role in the biologic manifestations of tumor promoter treatment [56]. In addition, a close correlation has been observed between activated c-Ha-ras and TGF-α expression in mouse skin tumors and epithelial cells [55]. Elevated expression of activated c-Ha-ras may also drive overexpression of EGFR or, alternatively, high TGF-α levels may lead to autoinduction of the EGFR. Regardless of the precise mechanism, elevated levels of the EGFR and its principal ligand, TGF-α, are strongly correlated with the development of neoplasias.
Many studies using the two-stage carcinogenesis model in mouse skin, as well as other tumor models, have identified dietary components which act through diverse mechanisms to alter the processes of tumor promotion and progression. Several examples are listed in Table 1. A number of retinoids, particularly all-trans retinoic acid, are specific inhibitors of TPA-tumor promotion in the mouse skin [57, 58]. Although their mechanism of action is not fully understood, evidence indicates that retinoids affect epithelial differentiation and also reduce elevated polyamine levels by inhibiting the induction of epidermal ornithine decarboxylase [59]. Recent evidence also indicates that retinoids bring about many of their effects by interacting with nuclear receptors. These nuclear receptors are trans-activating factors that can regulate the expression of specific genes involved in differentiation, proliferation, and apoptosis [60, 61]. The synthetic retinoid fenretinde, which has shown promising chemopreventive activity against several cancers, appears to exert its antitumor effects primarily by inducing apoptosis in damaged cells [62]. It has become clear in the past few years that apoptosis and mitogenesis are equally important processes in maintaining cell number homeostasis; thus, in addition to cell proliferation, apoptosis has emerged as a critical target for prevention.

One of the clearest examples of dietary modulation of skin carcinogenesis through alteration of the PKC pathway comes from the laboratory of Birt et al. [63] showing that calorie restriction, which inhibits skin tumor promotion by TPA, inhibits PKC activity and decreases the amount of specific PKC isoforms (particularly PKCα and PKCζ). Birt et al. [64] have also shown that feeding high corn oil diets increases PKC activity in epidermal cells, apparently by modulating epidermal cell lipid metabolism rather than altering specific PKC isoforms.

**Inflammation**

In addition to inducing changes in gene expression by activating specific signaling pathways, tumor promoters can elicit the production of protein factors such as IL-1 and several nonprotein factors through intracellular activation mechanisms [49]. Of critical importance to the promotion process is the release of arachidonic acid and its metabolism to eicosanoids [65]. Eicosanoids, which include the prostaglandins and hydroperoxy forms of arachidonic acid, are involved in such processes as inflammation, the immune response, tissue repair, and cell proliferation.

Prostaglandin synthesis is regulated by cyclooxygenase (COX) gene expression. Two separate gene products, COX-1 and -2, have similar COX and peroxidase activities, although they are differentially regulated [63, 66]. While a variety of factors, including serum, growth factors, and phorbol esters, can upregulate the mRNA levels of both COXs, the COX-2 gene generally responds in a much more dramatic fashion and thus has been referred to as a phorbol ester-inducible immediate early gene product [65].
A number of prostaglandin synthesis inhibitors are effective in counteracting skin tumor promotion and carcinogenesis. Compounds such as the anti-inflammatory steroids (i.e., glucocorticoids) are potent inhibitors of mouse skin tumor promotion by phorbol esters [49]. These compounds are effective phospholipase A₂ inhibitors, which could account for their ability to decrease the amount of arachidonic acid available for metabolism to important pro-inflammatory end-products. Inhibitors of the COX pathway, such as indomethacin and flurbiprofen, best studied as colon cancer chemopreventives [67], also inhibit skin tumor promotion at moderate to high doses in most mouse strains [49]. The COX pathway has recently become a major prevention target, primarily due to the role of these enzymes (particularly COX-2) in inflammation, as well as in apoptosis and cellular adhesion in some cells [68]. In addition, several safe, effective and inexpensive agents that can perturb the pathway, such as the nonsteroidal anti-inflammatory drugs, are readily available, and several food components with COX-inhibitory activity, such as resveratrol [26], are being identified.

**Immune Surveillance of Malignant Cells**

Cancer patients often have impairments in several components of their immune systems, such as tumor-infiltrating lymphocytes, circulating T cells and macrophages. Tumors can release immunosuppressive components, thus having a negative impact on the immunosurveillance system. However, immunotherapy (involving enhancement of tumor cell killing through administration of cytokines, vaccines, or immune cells) has demonstrated great potential against certain cancers, and some nutrients (vitamin A, ω-3 fatty acids) may also enhance antitumor immunosurveillance [69]. We and others have shown that the proliferative responsiveness of splenocytes to mitogens such as the plant lectins, concanavalin A and phytohemagglutinin, and the bacterial cell wall component, lipopolysaccharide, is consistently enhanced by calorie restriction [70, 71]. Calorie restriction also reportedly enhances the production of IL-2 by splenocytes and T cell-mediated cytotoxicity [72]. NK cell activity, especially when induced by poly I:C, is also stimulated by calorie restriction [73]. NK cells represent a subpopulation of lymphocytes that mediate cytotoxicity against target cells, particularly tumor cells, that is not restricted by the presence of major histocompatibility antigens. Thus, calorie restriction provides an example of a dietary manipulation that enhances immune competence, although the role of the immune system in the anticancer effects of calorie restriction and other dietary perturbations remains unclear and should be an area of focus in future studies.

**Tumor Progression Processes**

As noted earlier, the process of tumor progression is largely an extension of tumor promotion involving the accumulation of additional genetic alterations in an initiated cell clone, which generally leads to a growth advantage for the progressing clone. Ultimately, progression leads from a focal lesion consisting of a
population of initiated and promoted cells to an invasive malignant tumor mass. One frequently observed genetic alteration that appears to contribute to malignant progression is mutation in the p53 tumor suppressor gene [74]. The p53 gene product is a transcription factor that regulates the expression of a number of DNA-damage and cell cycle-regulatory genes, as well as genes regulating apoptosis. By enhancing transcription of these critical genes, p53 regulates the growth response after DNA damage [75]. p53 also plays a role in maintaining genomic stability [76]. A hallmark of spontaneous malignant progression, genomic instability is characterized by sequential chromosomal aberrations such as duplications, deletions and loss of heterozygosity which lead to rapid accumulation of unfavorable genetic alterations and eventually to malignant cell growth. Cell number homeostasis, normally maintained through a balance of genes regulating cell proliferation and apoptosis, is lost. DNA hypomethylation, frequently observed in malignant tumors, may also contribute to malignant progression [77]. Thus, p53 and other cell cycle and apoptotic regulators, as well as other genes regulating genomic instability and DNA methylation, are critical targets for prevention strategies at this late stage of the carcinogenesis process. The angiogenesis process is also being identified as a critical prevention target in the promotion/progression stage. At present, very little is known about the nutritional modulation of angiogenesis, although calorie restriction has recently been shown to inhibit angiogenesis [78]. Thus, angiogenesis should also become an area of focus in studies addressing the relationships between dietary factors and cancer prevention.

**Future Prospects: Transgenic Mouse Models for Nutrition and Cancer Prevention Studies**

Future progress in nutrition and cancer prevention research may be facilitated by the use of animals with specific genetic susceptibilities for tumor development. As mentioned previously, the majority of chemically induced tumor models used to date in carcinogenesis research were developed prior to the identification of most of the currently known cancer-related genes. The major goal in the development of these carcinogen-induced tumor models was the rapid generation of neoplasia to provide investigators with sufficient material in a timely fashion for studying tumor formation. These models generally involve high-dose regimens of a single genotoxic carcinogen which can induce large-scale genetic damage, often in a random fashion. Although some of the molecular alterations have been identified in the commonly employed models, the types of alterations caused by high-dose chemical exposure do not generally reflect the gene-environment interactions underlying the pathogenesis of cancer in humans. Furthermore, the interpretation of the activity of preventive compounds being evaluated in these models can often be confused by the effects of those compounds on the metabolic
activation or detoxification specific to the high dose of carcinogen, which may or may not be mechanistically relevant for typical human exposures to exogenous or endogenous carcinogens.

The recent development of mouse strains with carcinogenesis-related genes overexpressed or inactivated provides investigators with new models for studying the carcinogenesis process and for testing strategies to offset specific genetic susceptibilities to cancer. p53-knockout mice, which my group has focused on, provide an excellent example.

Mutation of the p53-tumor suppressor gene is the most frequently observed genetic lesion in human cancer; over 50% of all human tumors examined to date have identifiable p53 gene point mutations or deletions [79]. Donehower et al. [80] first reported in 1992 that homozygous p53 knockout (p53–/–) mice were viable, but highly susceptible to spontaneous tumorigenesis (particularly lymphomagenesis) at an early age. p53–/– mice have been useful tools for studying the role of p53 in carcinogenesis. For example, in response to the two-stage skin carcinogenesis protocol, p53–/– mice have the same frequency of benign papillomas as do wild-type (p53+/+) mice, but the papillomas progressed to malignant carcinomas much faster [81]. Furthermore, the carcinomas in the p53–/– mice were histopathologically more malignant, further confirming the importance of p53 loss in the acceleration of tumor progression. These mice are also an attractive and relevant tumorigenesis model for studying the nutritional modulation of cancer, given the frequency of p53 mutations in human tumors and the rapidity with which spontaneous tumors develop.

We have evaluated [82–85] the ability of several dietary and chemopreventive interventions to offset the increased susceptibility of p53–/– mice to spontaneous tumorigenesis, including calorie restriction. In p53–/– mice a 40% restriction of carbohydrate calories results in a 75% delay in the development of spontaneous tumors (which are mostly lymphomas in these mice) and a slowing of the traverse of lymphocytes through cell cycle [82]. The time to tumor onset in these mice is p53-dependent: most p53–/– mice develop and die from spontaneous tumors by approximately 6 months of age, compared with nearly 2 years for p53+/+ mice. However, the highly statistically significant tumor-delaying effect of calorie restriction, relative to ad libitum feeding, is similar in both p53–/– and wild-type mice, indicating that the mechanisms underlying calorie restriction may be independent of p53 [84].

Knockout mice which are completely deficient in p53 (p53–/–) have also been useful for elucidating how calorie restriction and other interventions inhibit tumorigenesis. For example, the antitumor effect of dehydroxyepiandrosterone (DHEA) in p53–/– mice is independent of its effects on food intake or on nucleotide pool levels [85] as was previously suggested in studies using other animal models [86]. We showed [87] that both calorie restriction and DHEA decreased the rate of thymocyte proliferation, whereas DHEA, but not calorie restriction, increased the rate of apoptosis. The apoptosis-inducing effects of the chemopre-
ventive steroids appear to be mediated by decreased Bcl-2 gene expression. In addition, we showed [88] that calorie restriction, DHEA, and fluasterone each reduce nitric oxide levels and downregulate nitric oxide synthetase expression.

Numerous mouse models with inactivated or overexpressed cancer-related genes other than p53 have been developed in recent years [89–91]. The number of transgenic and knockout strains is too great to list here in a meaningful way; however, there are several recent reviews in the literature, as well as on-line databases of transgenic and knockout mice, such as the Induced Mutation Registry (IMR) database maintained by the Jackson Laboratory (www.jax.org/pub-cgi/imrpub) and Tbase, maintained by Biomednet (www.bis.med.jhmi.edu/bio/HGET/tbase2). As recently reviewed [89], many of these knockout and transgenic mouse models have been used effectively in toxicology and carcinogenesis studies, and may provide useful tools for the identification of key pathways involved in the modulation of cancer by dietary factors.

Conclusions

The purpose of this chapter is to provide an understanding of the potential targets for mechanism-based prevention strategies. Targets for interfering with tumor initiation events include: (1) modifying carcinogen activation by inhibiting the enzymes responsible for that activation; (2) enhancing carcinogen detoxification by altering the level or activity of detoxifying enzymes; (3) improving direct scavenging of DNA-reactive electrophiles, and (4) enhancing DNA repair processes. Also depicted in Figure 1 are targets for blocking the processes involved in the promotion/progression stages of carcinogenesis, including: (1) scavenging ROS, which in addition to acting at the initiation stage to damage DNA can also act at later stages to alter cell-signaling processes; (2) altering the expression of cancer-related genes, such as oncogenes and tumor suppressor genes; (3) decreasing inflammation; (4) suppressing proliferation, and (5) encouraging apoptosis. Also, additional targets, such as antitumor immune surveillance and angiogenesis, should be evaluated in future studies. Examples of dietary factors and chemopreventives interacting with each of these processes was provided.

Experimental models of carcinogen-induced cancer have been crucial to advancing our understanding of the influence of diet on chemical carcinogenesis, and future progress may be facilitated by the integration of genetically altered animals into cancer prevention studies. The utility of these mice for prevention research was discussed. An additional promising area for future studies may also be the use of multiple agents, particularly combinations of agents that work on different stages (i.e., agents that work chiefly at the promotion stage in combination with agents that block initiation events) or on different mechanisms within the same stage (i.e., inhibitors of proliferation along with anti-inflammatory agents).
In conclusion, progress in the field of carcinogenesis has revealed multiple targets for the nutritional modulation of cancer, and additional targets are being identified as we learn more about the molecular events involved in the carcinogenesis process. We must now translate this knowledge base, as well as capitalize on the availability of new tools, such as transgenic mice, to further our understanding of the ways dietary factors can effect carcinogenesis, and to develop and test new strategies for preventing cancer.

References


Nutrition and Carcinogenesis


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Discussion

*Dr. Mason:* In the energy restriction model, how certain are we that energy restriction is not simply retarding clonal expansion of an established neoplastic clone? In other words, that it is just retard ing the appearance of a tumor that’s going to appear eventually anyway? If this is true, the validity of the model would be in question.

*Dr. Hursting:* The energy restriction mechanism is difficult to elucidate because it does so many things. It does indeed act as an anti-initiating agent, but its primary effects are at the promotional phase and it very well may be that its main effects are just to slow things down. We’ve run this regimen through six or seven models, and not only does it affect the spectrum of tumor development, but there is a tremendous delaying effect. To my mind that’s not a
bad thing. The fact that it does not prevent the formation of tumors but can delay them by 100 or 200% could be extremely beneficial. If we can slow down latent prostate tumor development, for example, we may be able to ensure that it never becomes an invasive carcinoma. That, to my mind, is prevention. What we’re preventing here is not cancer; we are preventing the carcinogenesis process from evolving to its endpoint, which is invasive carcinoma. I believe the primary effect of obesity is to drive proliferation. However, I think it’s still very important to understand how energy restriction slows things down.

Dr. Guesry: I think the concept of energy restriction may be misleading. When you restrict food intake, you don’t only reduce total energy intake, you reduce fat intake and protein intake and so on. I think we would all agree that fat restriction plays a role in breast and prostate cancer, and that limitation of certain amino acids could have direct effects on tumor growth. Thus I think it is important to specify precisely what type of food restriction is involved.

Dr. Hursting: In our animal models we have always been careful to ensure that there is no change in fatty acid or amino acid intake. Our restrictions have always been purely of carbohydrate energy. Of course there are changes in the diet composition but we can control for those. Obviously in humans it is much more difficult to do this. Studies have been done comparing the effects of restrictions of carbohydrate energy, fat energy, and protein energy [1–3], but in our animal models such qualitative differences do not seem very important for the outcome – though perhaps the sharpest effect seems to be with carbohydrate restriction. However, if you give carbohydrate ad libitum and restrict lipid, you also get a nice anticancer effect, and same with protein restriction. So I believe the effect is mediated by total energy intake. I also want to emphasize that it’s not just the intake of energy, which is what we have been primarily involved in with our animal models, because it’s easy to do. The same effect can be produced by increasing energy expenditure. If you let animals consume food ad libitum but put them on exercise regimens so that you control their weight, that is also effective. So I think it is the energy flux that matters. I believe what is driving the effect is an anti-obesity intervention. I am somewhat concerned, although not greatly, that changes in diet quality may have played a role in our studies. For example, we have been rigorous in ensuring that there is no change in carotenoid intake. However, this doesn’t mean that the diet is not different in some way. Restriction of any component of the diet, carbohydrate in this case, will result in a relative increase in the density of the other components. That effect has been looked into so often that I believe we can rule it out as an issue here. The major effect really seems to come down to the matter of energy flux and the prevention of obesity. In my country, at least, obesity has become an epidemic, particularly among adolescents; this is one of the major areas to focus on in terms of cancer prevention.

Dr. Bloch: Could you comment on the percentage of the energy derived from carbohydrate, protein, and fat in your low-carbohydrate diet, and on the kinds of amino acids and fatty acids in the diet?

Dr. Hursting: I don’t have those numbers, but the diet is 50% sucrose with 5% corn oil as the lipid source. Basically we take out the sucrose and corn starch and replace them with an inert carbohydrate that is not metabolized. We adjust the diet of the energy-restricted animal so that when we restrict them to 60% of what the ad libitum group eats on a daily basis, that 60% provides exactly the same amount of amino acids and fat as the diet of the ad libitum group.

Dr. Yip: You have shown that diet does retard carcinogenesis, but does it reverse the process? In Malaysia we have a situation where after a diagnosis of cancer the non-medical nutritionists move in and put the patients on weird diets full of selenium, vitamins A, C, and E, and so on, and also exclude all meat from the diet. I find it hard to believe that this sort of diet could reverse the process.

Dr. Hursting: Other speakers will deal with this later in the Workshop. For my part, I’m not a big fan of major alterations in the diet as a therapeutic approach. I don’t think we know
a lot about how these changes will affect tumors once they’re formed. I’ve often been asked whether it is alright to continue using megadoses of isoflavones when one is on tamoxifen for breast cancer. That’s a real concern. We don’t know the answer, but it is an important issue. It’s clear that some dietary factors have important activities. There seems to be evidence that genistein and some of its metabolites are as anti-angiogenic as some of the existing pharmaceuticals [4] but the isoflavones also have estrogenic activity. So it’s clear that many of these dietary factors have important influences.

Dr. Go: I’d like to make your animal model relevant to human disease if possible. Clonal expansion, clonal selection, and intraepithelial tumor formation is a long process in humans, 20–30 years at least. Assuming that your energy-restriction models work, have you imposed restrictions cyclically, which might better mimic the human situation?

Dr. Hursting: There’s some old literature on fasting, where intermittent restriction has been shown to have some effects [5, 6]. It was found that during the fasting process a very strong pro-apoptotic pulse actually occurs about 12 h into a fast in a preneoplastic liver model. How relevant that is to other models we weren’t sure, but we are certainly interested in the possibility of cyclic restriction. Nobody wants to chronically restrict their dietary energy by 40%; that’s going to be a very difficult thing to sell. But if cyclic fasting could be shown to be effective it would be a practical possibility. We applied this to some of our models and found that though intermittent fasting was not quite as effective as chronic restriction it was still clearly effective, particularly in our mammary model. We found that if you fast the animals 1 day a week and then let them eat fully on the other 6 days, they compensated by overeating and there was no effect. So we restricted the animals for 6 days a week to the mean daily intake of the group, and then on the 7th day we withheld their food. This was effective to the extent that the survival curve was not far off that seen with the chronic energy restriction. So I think it’s promising, and certainly in human terms more appealing than overall chronic restriction. One thing that has been a little disappointing is that we’ve also tried a number of combination approaches where we’ve used energy restriction on top of DHEA or some of the analogs we’ve been studying, or genistein, and we haven’t seen any enhancement. In fact, these animals do less well, perhaps because those agents tend to induce food aversion.

Dr. Heimburger: How much do we need to re-interpret earlier literature on chemopreventive agents with regard to the possibility that they put the animals off their food, thus resulting in an unintended energy restriction? Perhaps what some of those beneficial chemopreventive agents were really showing was an energy effect. Our group is involved with redesigning some of the earlier retinoid and other chemopreventive agent trials in order to force isocaloric, isonutritional feeding between the treatment and control groups. Could you comment on that?

Dr. Hursting: I think that’s very important. These agents may cause taste aversion and have metabolic effects that reduce appetite, and if you don’t have a pair-fed group and if you’re not careful with the diets, you may have important misinterpretations. We had one study in a carcinogenicity testing program where carcinogenic agents appeared to be decreasing the background spectrum of tumors, and we found that these animals had become anorexic and reduced their feed intake by around 20%. That was enough to delay the main background tumor. The pressure is on us now to begin to move these trials into the human sphere, but in some cases we only have the animal data to guide us, so we need to be very careful with our models and with our diets and interventions.

Dr. Karupaiah: There’s been a lot of interest over the years in energy restriction and delayed aging. Do you have any comment about whether energy restriction may be delaying cellular aging in carcinoma cells?

Dr. Hursting: Yes, it was the aging data that stimulated the research into energy restriction and cancer. There are sufficient primate data now to show that energy restriction does seem to prolong lifespan and this is a real effect that can be translated between species. As to
the mechanism, there are many things that energy restriction does because it induces a big alteration in metabolism. For example, the animals are under stress so the glucocorticoid pathway is upregulated, while the growth hormone-IGF axis is decreased, and both of these can affect events related to aging. Inflammatory processes appear to be decreased, and that may be a major component. How these all work together is where the field is now. There’s no coherent picture as yet.

Dr. Riboli: That energy restriction works in animal models was known as long ago as 1914, when the first data on transplanting tumors were published [7], and then Tannenbaum [8] confirmed this in the 1940s, so it is very well established in animal models. There are animal models that you cited briefly which are much more relevant to the human situation than chronic severe energy restriction. For example, studies published in the early 1950s by Tannenbaum, in which animals were kept at low temperature, showed that the energy expenditure for maintaining thermoregulation was equally effective in reducing cancer occurrence. Epidemiologists are most interested in the only model that seems relevant in humans, which is the balance between energy intake and energy expenditure. In fact, if we look at all the prospective studies done in the 1950s and the 1960s, the humans who survived longest are those who eat more, not those who eat less! This is probably related to energy expenditure, because the best correlate of energy expenditure as physical activity is energy intake. So a more interesting way to direct animal models would be to look at the ratio between energy intake and energy expenditure/physical activity. Our group is particularly interested in how the balance between energy intake and physical activity may influence insulin and IGF activity and bioavailability, and these may be important in tumor growth. Incidentally, as an epidemiologist I don’t mind whether the carcinogenic process is completely inhibited or just very delayed. I think we would all be quite happy to have a tumor at age 150!

Dr. Hursting: I agree. We don’t need any more studies to show that this works. We need to understand how it works and how it links in with what’s going on in the human.

Dr. Argilès: There are several models where energy intake is greatly increased, and energy expenditure is reduced, such as the cafeteria diet model. In those conditions, what do we know about the incidence of tumors?

Dr. Hursting: In some of those models, there does appear to be tumor enhancement. We are interested in the possible interactions with IGF and leptin in this situation.

References