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Risk Factors for Colon Neoplasia—Epidemiology and Biology

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Epidemiological, physiological and molecular models of colon carcinogenesis have been proposed. Consistent epidemiological risk factors include reduced plant-food intake (increased risk); elevated meat intake (increased risk); higher physical activity (reduced risk); and increased alcohol intake (increased risk). At the physiological level, these lifestyle variables may trigger processes that provide explanations for the associations: higher meat, fat and alcohol means more heterocyclic amines and higher levels of bile acids; higher plant food means higher intake of several anticarcinogens and fibre fermentation that produces volatile fatty acids; exercise has a variety of beneficial effects. This complexity is elaborated further in the context of the colonic milieu where interactions among digesta, bacteria and epithelial cells occur. The long-term likelihood of cancer is the summation of moment-to-moment changes in the colonic milieu brought about by this interaction. Possible relationships between established epidemiological risk factors, genetic susceptibility and somatic genetic changes are outlined.

Key words: colon, cancer, neoplasia, diet, fat, meat, vegetables, genetic susceptibility, epidemiology, molecular biology

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INTRODUCTION

INCIDENCE RATES of colon cancer vary approximately 20-fold internationally. The highest rates are seen in the developed world–North America, Western Europe, Australia—with age-adjusted (world standard) incidence rates of 25–35 per 100 000 in the late 1980s. The lowest rates are seen in India (1–3 per 100 000) [1–3]. Colon cancer is the only cancer that occurs with approximately equal frequency in men and women [4]. Migrant studies, as well as the recent rapid changes in incidence rates in male Polynesians in Hawaii and in the populations of Italy and Japan, demonstrate that the disease is sensitive to changes in environment. However, colon cancer has long been known to occur more frequently in some families [5], and there are several rare genetic syndromes which carry an excess risk of colon cancer [6–8]. This is, thus, a disease for which there is both a genetic predisposition and some causal environmental exposures.

The review will consider the epidemiology of colon cancer and briefly relate it to what is currently known about the physiological and cellular and molecular biological mechanisms involved in large bowel carcinogenesis. The approach taken, developed in detail elsewhere [9], is the idea of the aetiology of chronic disease in general, and colon cancer in particular, as being interpretable via several different models, involving epidemiology, physiology, and cellular and molecular biology. These models are not reducible to the most basic, but rather are interrelated in complex ways, each providing information for the other and ultimately improving both our understanding of the disease and our capacity to implement approaches to prevention and early detection (Figure 1).

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DIET AND COLONIC NEOPLASIA—EPIDEMIOLOGY

The idea that dietary fat is causal in colon cancer had its origins in several observations: international correlation studies

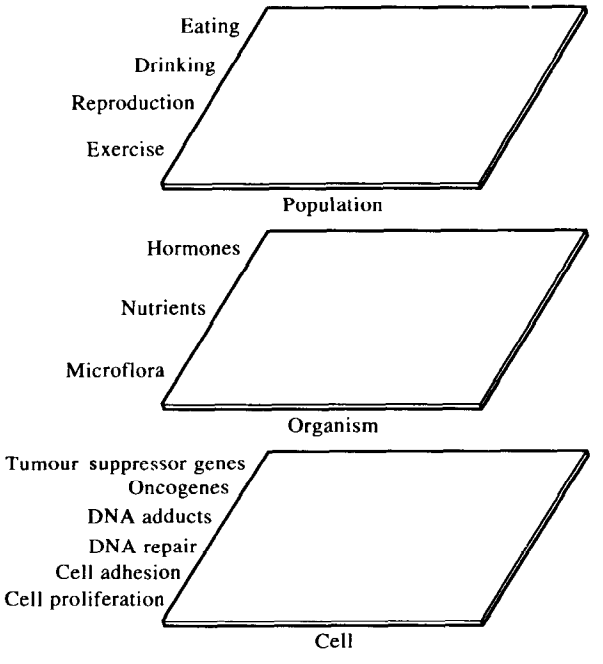

Figure 1. Multilayered model of a colon cancer causation.

[10], the metabolism of bile acids [11], and some early case–control studies of diet and colon cancer [12, 13]. There are now almost 30 case–control and cohort studies that have explored the association of cancer risk with meat, fat or protein consumption, and approximately two-thirds of these have shown a positive association between risk and higher intake; very few show an inverse association [14]. As examples, Haenszel's 1973 study of Hawaiian Japanese found a 2.5-fold increase in risk for those consuming beef 12 or more times per month compared with those eating it less than eight times per month [12]. The Toronto study, in 1980, reported an almost identical increase in risk for those in the highest tertile of saturated fat consumption versus the lowest [15]. The Adelaide study in 1986 [16] found an almost 4-fold increase in multiply-adjusted risk for those women in the highest quintile of protein consumption versus the lowest. Willett and associates reported an almost 2-fold increase risk for the highest quintile of red meat consumption in the Nurses' Study [17]. Taken together, there data strongly suggest that some aspects of a diet high in meat, fat and protein (these are all highly correlated) are relevant to the aetiology of colon cancer. Four studies have shown an inverse association with fish or seafood [14].

A role for dietary fibre in colon carcinogenesis was first proposed by Burkitt in 1969 [18]. The idea stemmed from Burkitt's clinical observation that colon cancer (and other disorders) were rare in Africans whose diet was high in unrefined foods. The more impressive and consistent lower risk, however, has been associated with the consumption of vegetables. Of the 29 studies that have published findings for vegetables, 24 have reported an inverse association [14]. Inverse associations with fruit are much less frequent. This is almost certainly not explained by the fibre content of vegetables alone, and may be only a particular manifestation of the broader association of vegetables with lower cancer risk [19]. One possible important dietary pattern is that described by Giovannucci and colleagues, who noted, in both polyps and colon cancer studies [20, 21], that a diet low in folate (a vitamin derived particularly from green leafy vegetables), especially in the presence of low methionine and high alcohol, is associated with an elevated risk. Nonetheless, the inverse association with fibre is almost certainly relevant and was most well indicated in the meta-analysis of 13 case–control studies by Howe and associates [22]. In those populations where cereal consumption is high, however, it is puzzling that risk is higher in association with higher consumption of rice (Japanese) or pasta and rice (southern Europe) [14].

Adenomatous polyps are widely accepted as precursors of colorectal cancer in humans. These non-malignant neoplastic lesions have themselves been the subject of a number of studies since 1986, when Hoff and colleagues published research [23, 24] on risk factors thought to be important in the aetiology of colon cancer itself. There are a total of 10 studies in the literature where the roles of diet and alcohol in the aetiology of adenomatous polyps have been addressed [25]. As with colon cancer, the most consistent association is with plant foods, with carbohydrate, and with fibre; eight of nine studies reported a lower risk for one or both sexes [25]. Two of two studies reported lower risks for higher folate intake, and three of three found physical activity to be inversely associated with risk. Positive risk factors are less consistent; four of seven studies reported higher risks in association with higher fat intake, although the types of fat were not consistent. Three of five reported higher alcohol intake in cases than controls [25]. Smoking is consistently associated with increased risk—a finding that is not common in colon cancer itself [25].

Thus, the original fat-and-fibre hypothesis may be equally well thought of as the meat-and-vegetables hypothesis. Indeed, Manousos and colleagues [26] have shown an 8-fold variability across the spectrum of high meat/low vegetable to low meat/high vegetable intake.

Other aspects of diet appear also to be independently related to colorectal cancer risk—particularly calcium intake and perhaps alcohol consumption [14]. There are also data to suggest roles for physical activity [27–30], gender and reproduction [14, 31–35]. The aetiology of bowel cancer, as the epidemiological evidence appears to suggest, may be a consequence of a variety of interacting environmental factors on a background of greater or lesser genetic predisposition or it may be, of course, that the aetiology is relatively simple but the right question has evaded us.

PHYSIOLOGICAL AND BIOCHEMICAL MODELS

The earliest mechanistic model of colon carcinogenesis was the bile acid hypothesis [36]—modified by an increasing understanding of colonic fermentation, volatile fatty acid production, and a variety of resultant anticarcinogenic effects [37, 38]. More recently, the effect of cooking on food, resulting in both potential carcinogens such as heterocyclic amines [39] and promoters [40], combines the old search for human gut carcinogens with some new ideas.

These physiological, biochemical hypotheses are neither mutually exclusive nor incompatible with the epidemiological observations. Specifically, fat increases bile acid production, ultimately increasing the exposure of the bowel mucosa to the toxic, trophic and promoting effects of (particularly secondary) bile acids [36]. Fibre binds bile acids, reduces transit time, increases stool bulk, ferments volatile fatty acids which may be directly anticarcinogenic [37] and which, by lowering pH, may reduce the conversion of primary to secondary bile acids [38]. Thus, the meat-and-vegetables hypothesis and the bile acid/volatile fatty acid hypothesis appear to present a one-to-one mapping of epidemiological on physiological models.

The cooked food hypothesis proposes, however, that the fat explanation is incomplete. High-fat diets contain greater amounts of heterocyclic amines from meat proteins [39] and promoters as a consequence of cooking at a higher temperature (cooking in fat produces higher temperatures than cooking in water) [40]. Thus, argue the proponents of this idea, the meat-and-vegetables hypothesis is really the high temperature:increased carcinogens/low temperature:low carcinogens hypothesis. These, again, are not incompatible models and, rather than simplifying the picture, may provide evidence for a complex, interactive aetiology, where one environmental exposure may act at several stages in the carcinogenic process.

Vegetables, particularly, have a variety of anticarcinogenic properties at both physiological (via stool bulking, transit time, etc.) and molecular levels (see below) [41–43].

Calcium, it has been proposed, lowers risk by binding bile acids and fatty acids, thereby reducing exposure to these toxic, trophic and cocarcinogenic compounds. Additionally, calcium may reduce cell proliferation directly [44]. To date, evidence for these mechanisms from human studies is scarce.

One other aspect of gut physiology is worthy of consideration—endocrine metabolism. There is substantial evidence that subsite and age differences in colon cancer risk vary by sex [31, 45]; there is a female excess of right-sided colon cancers at

all ages and a male excess of left-sided cancers. There is evidence, also, of increased risk in association with having fewer children [14, 32, 33] though this, intriguingly, is sometimes [33], but not always [46], different between men and women! Further, there are physiological correlates for these observations: men and women differ, under controlled experimental conditions, on transit time, stool bulk, volatile fatty acid production and bile acid production; the differences are, by and large, consistent with the epidemiological observations [47–49]. These data suggest differences in gut metabolism between the sexes that may be mediated, ultimately, by hormones, and may also suggest differences in colonic bacterial populations, fermentation rates and general colonic milieu.

At epidemiological and physiological levels, our current models of the aetiology of colon cancer are relatively consistent. However, the influences of fat, fibre, calcium and vegetables, cooked food, reproduction, alcohol and exercise each seem to work through multiple and different but, in some cases, overlapping pathways to carcinogenesis. The variety of exposures—ingested foods and host responses and states—primarily determines the environment to which the colonic cell is exposed, i.e. these factors determine and condition the growth media [9, 50], both luminal and plasma, in which the colonic cells are bathed. Thus, each of the epidemiological risk factors operates through a variety of pathways that can reasonably be thought of as physiological cascades [9]. For example, meat and fat are a source of DNA-interacting carcinogens and increase hepatic bile acid production. Other factors, including bacterial species, bacterial enzyme production and transit time will alter the production and concentration of secondary bile acids, thereby modifying the process of cell damage and repair, and causing changes in gut surface area. The increase in cellular workload will increase cell replication rates.

In contrast, ingested vegetables increase the fibre content of the large bowel, which may bind bile acids or may undergo fermentation, thus increasing both bacterial mass and volatile fatty acid production. This, in turn, produces effects on cell replication and cell maturation and inhibits secondary bile acid production by reducing intraluminal pH. Meanwhile, the fibre and the increased bacterial mass increase stool bulk and increase the work-load of gut musculature and reduce transit time.

On the plasma side of the colonic cells, there is not only the opportunity for exposure to carcinogens and anticarcinogens, but also the likelihood that specific relationships—both accelerating and controlling growth—exist between fibroblasts and epithelial cells; these may be particularly mediated by endogenous growth factors [51, 52]. The recent findings suggesting lower risk in association with non-steroidal anti-inflammatory drugs (NSAID) use provide empirical evidence for this mechanism [53]. Whether growth factor paracrine/autocrine loops are also related to some of the other established risk factors is a reasonable and testable question [54].

Overall, risk of colon cancer, under this model, can be regarded as the summation, over long periods of time, of the moment-to-moment outcomes of these physiological cascades and molecular loops [9].

CELL/CRYPT MODEL

The target cells of colon carcinogens are (probably progenitor) crypt epithelial cells. Thus, increasing attention has focused on the transition from normal to malignant cell and from macroscopically normal mucosa through adenomatous polyps to cancer. The adenoma/carcinoma sequence, as originally proposed by Hill and colleagues [55], is now the accepted description of this process, and one that allows intervention in those with polyps to substitute for large-scale, cancer prevention trials. However, the agents hypothesised by these authors to explain both international differences and the transitions in individuals from one state to the next remain to be elucidated.

A likely marker for increased adenoma/carcinoma risk is a persistently rapid cellular replication rate [56]. Again, this is important because, if indeed the hypothesised adenoma/carcinoma sequence is preceded by a normal cell/adenoma sequence, even smaller and more rapid tests of the anticarcinogenic potential of specific agents (and even behavioural strategies) become feasible. One other "stage" in this process has been proposed—the aberrant crypt or the microadenoma [57]; its appropriateness for human studies is not yet established, but it has been used as an endpoint in animal studies [40], and may represent an intermediate in the transition from normal cell to adenoma.

There are two lines of inquiry that can be integrated with the microanatomical changes described above. The first is work at the molecular level that provides some confirmatory evidence for the coherence of, and even mechanisms for, the adenoma/carcinoma sequence. The second, perhaps even more interesting, is whether there are any data to tie the epidemiological risk factors (particularly the dietary exposures) to the molecular and cellular events.

MOLECULAR MODELS—GENETIC PREDISPOSITION

One link across genetic susceptibility, diet and DNA-damaging compounds is represented by exposure to, and metabolism of, arylamines. These compounds are present in large amounts in cooked protein; their metabolism (and therefore the extent to which colonic DNA will be exposed) is under genetic control. The mechanism, as proposed by Kadlubar and colleagues, appears to be as follows: heterocyclic amines readily undergo hepatic *N*-oxidation (itself a function of the activity of the phenotypically polymorphic enzyme, $P450_{1A2}$) and subsequently *N*-glucuronidation. These conjugated *N*-hydroxy metabolites are transported to the colon, deconjugated by bacterial β-glucuronidases and reabsorbed. In the epithelial cells, the *N*-hydroxy derivatives are good substrates for *O*-acetylation (also via a polymorphic enzyme—NAT2), producing *N*-acetoxy arylamines which readily form DNA adducts [58]. Kadlubar and colleagues have recently shown that both NAT2 status and $P450_{1A2}$ status predict risk, and that fast acetylators who are also fast *N*-oxidisers (approximately 16% of the North American population) are at nearly three times the risk of those who are slow acetylators and slow *N*-oxidisers [59].

Familial adenomatous polyposis (FAP), also called adenomatous polyposis coli, is a rare genetic syndrome [6, 7]. It is characterised by the development, as early as childhood, of multiple adenomas, numbering from a few polyps to several thousand. FAP carries an almost 100% risk of colorectal adenocarcinoma. Localisation of the FAP gene—*APC*—was independently accomplished by Leppert and associates and Bodmer and associates in 1987 [60, 61]; the gene was mapped to chromosome 5q. Gardner syndrome was also shown to be linked to the 5q markers [62]. The relevant gene is now sequenced and a variety of germline mutations have been described [63–66]. Mutations at the *APC* locus are a common and probably a very early somatic event in polyps and cancer [67], supporting the idea that, for some individuals, the first hit is the germline mutation of the gene and, for others, it is an early somatic event.

The causes of the somatic events remain to be established. The protein product of *APC* associates in the cell with proteins called catenins [68, 69]. This provides evidence that *APC* is involved in some manner with cell adhesion and cell communication. Whether there are links to diet or other risk factors remains to be established.

Another form of colon neoplasia that shows familial aggregation is hereditary non-polyposis colorectal cancer (HNPCC) [70, 71]. This syndrome is not easily distinguished from sporadic polyposis and cancer on physical examination. The most clearly distinguishing features of the family history are the tendency to early onset and a pattern of other cancers—particularly those involving endometrium, the urinary tract, the stomach and the biliary system [71]. An analysis involving two large HNPCC kindreds [72, 73] found strong linkage to anonymous microsatellite markers on chromosome 2p15-16 [72] and suggested a mechanism different from that associated with the inherited abnormality of tumour suppressor genes, perhaps involving a predisposition to genetic instability and manifesting as widespread alterations in short repeated DNA sequences [73, 74]. Several such DNA mismatch repair genes are now known to exist in the human genome and have been linked to HNPCC [75].

MOLECULAR MODELS—SOMATIC EVENTS

Vogelstein and colleagues [76–81] have provided extensive evidence that there is an accumulating (but not necessarily linear) series of specific chromosomal and genetic changes that accompany (and perhaps cause) the transition from normal colonic mucosa to metastatic carcinoma. Further, some of these changes [79] show the importance of cell–cell communication at stages other than those involving *APC*, perhaps emphasising the relevance of cell adhesion both early in carcinogenesis (via disruption of cell communication or impairment of sloughing of cells at the luminal surface) and late in carcinogenesis (via changes in metastatic potential).

POSSIBLE LINKS BETWEEN EPIDEMIOLOGY AND MOLECULAR EVENTS

Are there any data to link the epidemiological risk factors to the molecular biology? Several lines of inquiry currently hint at relationships and may provide insights into the kinds of questions it is increasingly possible to answer.

DNA hypomethylation is an early step in colon neoplasia [82]. Methylation is under genetic control, and expression of the methyl transferase gene is considerably increased in the normal mucosa of cancer patients and is even higher in polyp and cancer tissue [83]. Of even greater interest to the discussion here is whether there are environmental influences on DNA methylation. Chronic methionine and choline deficiencies produce alterations in DNA methylation and a variety of tumours in rodents [84]. Folate deficiency may have related effects [85]. Giovannucci and associates [20, 21] have recently shown that high folate, low methionine and high alcohol (itself often associated with low folate) are all associated with increased risk of colonic adenomatous polyps and cancer, suggesting strongly that the abnormalities of methylation seen in early colon neoplasia are plausibly related directly to dietary factors. Do these data point generally to mechanisms by which substances without direct DNA-interacting capacity are, nonetheless, involved at a molecular level in the cancer process?

Certainly, there is evidence of other dietary influences at the molecular level: one of the most interesting is the hypothesis from Weinstein and colleagues that fat is important in colon carcinogenesis because it is a source of diacylglycerol (DAG) [86, 87]. These workers point out that intracellular DAG is an important part of the cascade that leads from *RAS* activation via a G-protein or from growth factors via receptors to protein kinase C activation, protein phosphorylation and cell turnover. They have proposed that the interaction of fat, bile acids and bacteria in the colon produces excess intraluminal DAG which will mimic and amplify these cell replication signals [86].

Vegetables contain a large number of substances—both micronutrients such as carotenoids and ascorbate, and other bioactive compounds such as phenols, flavonoids, isothiocyanates and indoles with a variety of potent anticarcinogenic properties [41–43]. The steps from procarcinogen to neoplastic cell replication or (re-)differentiation can be broadly considered as follows: procarcinogen is activated to ultimate carcinogen (either of which may be solubilised and excreted by a variety of P450 and phase II enzymes); carcinogen passes through membranes; carcinogen interacts with DNA; synthesis and replication of abnormal DNA (or DNA repair) occur; cell replication with abnormal DNA and abnormal protein synthesis (or cell differentiation or apoptosis) result. At almost every one of these steps, one or more known phytochemicals can alter the likelihood of carcinogenesis, usually in a favourable direction but sometimes in a way that enhances risk. For example, glucosinolates and indoles, isothiocyanates and thiocyanates, phenols, and coumarins can induce solubilising and (usually) inactivating enzymes; ascorbate and phenolic compounds can block the formation of carcinogens such as nitrosamines; carotenoids and flavonoids can act as antioxidants; lipid-soluble compounds, such as sterols and carotenoids, may alter membrane structure or function; some of the sulphur-containing compounds may suppress DNA and protein synthesis; carotenoids may suppress DNA synthesis and enhance differentation.

The fermentation of fibre results in the intraluminal production of volatile fatty acids, such as butyrate. Data from Paraskeva's laboratory have recently shown that, in colon tumour cells in culture, at least, volatile fatty acids will induce apoptosis—yet another possible explanation for the lower risk that a diet high in fermentable fibre may confer [88, 89].

It is also possible that dietary constituents could influence both early and later stages of the carcinogenic process via effects on gene expression. For instance, the level of dietary fat has been shown, in experimental animals, to alter both the production of eicosanoids [90, 91] (which, in turn, can influence DNA synthesis and tumour promotion) and the induction of genes coding for phase I and II metabolising enzymes [92, 93]. Contrary data do exist, however [94]. Further, fasting and refeeding are followed by structural changes in the chromatin at the site of genes involved in metabolic regulation, and the degree and kinds of changes are dependent on the amount of fat and protein in the diet [95]; this, via endocrine and paracrine stimuli, could have a major effect on cell metabolism and replication rate.

Dietary variables with the capacity for direct DNA damaging effects include arylamines described above [39]. It is worth noting that the transversion and transition mutations seen with a high frequency in *KRAS* are plausibly related to the interaction between arylamines and DNA [96]. To take one additional step, but one for which there are, as yet, no data, it seems plausible that the lesions resulting from interactions between arylamines and other carcinogens on the one hand and genomic DNA on the other, are more poorly repaired, or less frequently repaired, in those with abnormalities of DNA repair. This could provide

a direct link between diet and the germline abnormalities in DNA mismatch repair enzymes seen in HNPCC.

The suggestion of coherence among the epidemiology, the physiology and the molecular biology is encouraging and challenging. Perhaps more importantly, seeking such coherence across aetiological models is a process that may be able to be generalised to other diseases. Finally, and as noted at the beginning, it is worth bearing in mind that mine is not a reductionist position; it is rather that there are different levels of explanation that each cast light upon the others.

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