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Review

Dietary fibre and colorectal cancer: A model for environment – gene interactions

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As environmental factors are clearly associated with risk for colorectal cancer, we set out to model how dietary fibre, or the effects of its ingestion, might impact upon the complex events that characterise colorectal oncogenesis. The diverse nature of dietary fibre and its resultant fate in the gut is outlined. The evidence indicates that different types of fibre create different conditions in different regions of the gut. This is reflected in different effects on oncogenesis especially in animal models. Data from animal models show that insoluble fibre is protective. Evidence from human studies are not consistent, especially considering the interventional studies. However, all such studies have been dependent on biomarkers short of cancer formation, for measurement of an effect. The biological and molecular events characteristic of colorectal oncogenesis are reviewed in an effort to identify how fibre ingestion might regulate oncogenesis. While several mechanisms might account for protection, the results of fermentation and especially butyrate production provide examples of how genomic instability might be controlled. Activation of apoptosis and cell cycle arrest seem likely to be mechanisms that would enable correction of genomic events that drive oncogenesis. Butyrate itself can regulate gene expression by both epigenetic and direct effects.

Keywords: Colorectal cancer / Dietary fibre / Fermentation / Genomic instability / Review / Short-chain fatty acids

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Abbreviations: AARGC, acute apoptotic response to genotoxin-induced damage; **SCFA**, short-chain fatty acid

1 Introduction

Diet is clearly linked to both the risk for and incidence of colorectal cancer. It has been estimated that approximately 70% of the causation of colorectal cancer is due to dietary factors and that an optimal dietary approach might prevent this much of the disease [1]. In addressing the link between dietary fibre and colorectal cancer, we need to address sev-

eral major issues. The first concerns the impact – is dietary fibre protective? The possible link between colorectal cancer and consumption of dietary fibre has received considerable attention and a simple literature search will generate almost 1800 citations. What can we learn from this huge amount of information? The second is mechanistic – how does dietary fibre, its constituents, or its derived products, interact with the complex process of colorectal oncogenesis? Again, there is a substantial volume of literature on the subject and a simple search for papers linking colorectal cancer with fermentation, one possible mechanism of protection, generates almost 600 publications. Addressing the second point should not necessarily be delayed until we have the answer to the first. Unless we can better understand how fibre might interact with oncogenesis, we are not well placed to specifically design the types of studies that can address the question of protection. As will be described below, there are several quite different mechanisms by which dietary fibre might protect.

In an effort to constructively address this large body of information, we will use this review to model several relevant biological concepts: 1. The complex effects of dietary fibre on the colonic luminal environment. 2. The mechanisms by which dietary fibre, its constituents, its products or diet-generated conditions, might impact upon the complex genomic events that characterise colorectal oncogenesis. To develop these models, we first set the scene by providing an overview of background issues: (i) The nature of dietary fibre and what happens to it in the gut. (ii) The evidence that fibre protects against colorectal cancer. (iii) The biological and molecular events characteristic of colorectal oncogenesis.

2 The nature of dietary fibre

Fibre is homogeneous and is sourced from a broad range of different plant foods. There is no internationally accepted definition either in terms of the dietary sources, or the chemical nature of fibre [2]. Physiological, chemical, functional, epidemiological, and clinical definitions all have their place. Readers are referred to the review by Kim *et al.* [3] for a clinical perspective on the nature of dietary fibre.

In simple terms, dietary fibre can be considered to consist of complex carbohydrates that reach the colon. These complex carbohydrates include starch which resists digestion [4] and a range of non-starch polysaccharides (cellulose, hemicellulose, pectin, gums, and mucilages). This definition, while useful, ignores a major cell wall component, lignin, which is highly associated with these complex carbohydrates and might exert benefit. It also ignores other common minor constituents of the fibre component of foods, such as phytate, cutins, saponins, lectins, waxes, and other compounds.

The dietary source is relevant because different types of food contain different types of "fibre". This leads to a classification of fibre which is clinically and mechanistically useful. The soluble fibres, *i.e.*, the pectins, gums, mucilages, and some hemicelluloses, tend to be highly fermented in the proximal colon including the caecum. Little intact fibre from these sources reaches the rectum or is passed in the stool [3–6]. The relatively insoluble fibres, namely the celluloses, lignins, and most hemicelluloses, are more slowly fermented, have a greater impact on fermentation events in distal colon and can be readily detected in faeces [5, 6]. Sources of soluble fibres include fruit and oats while insoluble fibre is found in wheat.

It is obvious that any detailed consideration of the relationship between dietary fibre and colorectal cancer needs to take into account the difference in types of fibres, the different constituents of dietary fibre, and the different food sources in which they are normally consumed. This makes epidemiological studies extremely difficult. One can estimate food source intake but cannot measure actual fibre consumption.

Fibre intake is extremely variable throughout the world. The amount consumed clearly depends on the nature of the diet but estimates of consumption also depend on the method of analysis and whether or not the method of analysis includes a measurement of resistant starch, *i.e.*, starch which has escaped digestion in the small intestine [2]. A typical western diet might include fibre intake of up to 35–40 g per day and would generally average around 12–20 g per day depending on the method of measuring fibre consumption.

3 Fibre fermentation

Dietary and endogenous residues that reach the lower gut of animal species and the colon of humans may be metabolised by the anaerobic bacteria to produce short-chain fatty acids (SCFAs), plus lactate, ethanol, hydrogen, methane, and carbon dioxide. The process of breakdown of these residues is collectively known as fermentation [7]. The predominant substrates that contribute to the production of SCFAs are polysaccharides, oligosaccharides, protein, some sugars, and mucus. The principal SCFAs that result from carbohydrate fermentation are acetate, propionate, and butyrate and to a lesser extent isobutyrate, valerate, and isovalerate, which arise from amino acids. Bacteria in the colon utilise the energy released during the process of fermentation for their proliferation, which is accompanied by the synthesis of protein from peptide, amino acids, and ammonia [8].

Fermentation is regionally distributed in the colon. Normally it is most active in the caecum [9]. The major contributory substrate is probably resistant starch rather than non-starch polysaccharides [4, 10]. But different fibres are fermented in different regions [5, 6]. Insoluble fibre tends to ferment in distal colon [5] while soluble fibre ferments more proximally and more rapidly. This is an important distinction as the major burden of colorectal cancer is in the distal colon.

4 Evidence for protection in humans

The idea that increased consumption of fibre might be associated with reduced incidence for colorectal cancer was highlighted by Burkitt in 1970 [11]. Since then, several types of human epidemiological studies have been undertaken to address the possible relationship: correlative studies, case control studies, and prospective studies. Overviews of the findings from these studies have been presented by many investigators and a synthesis of these perspectives is presented. The methodology for assessing dietary intake has varied between these studies. They include the 24 h recall method, the three to seven day food diary method, and food frequency questionnaires [3].

4.1 Correlative studies in humans

In a consideration of 28 published international within-country correlative studies (see [3]), it was observed that 23 of these (82%) showed either a strong or moderate protective effect of dietary fibre or fibre-rich foods on colorectal cancer. Four studies found no significant effect and one showed a significant excess risk.

4.2 Case control studies in humans

Case control studies compare prior consumption of dietary factors in subjects with colorectal cancer and match them to control subjects without colorectal cancer. Obviously, accuracy is limited by tools for measurement of actual dietary consumption and within any given population, the range of consumption of dietary fibre might be narrow and hence the apparent effect small. Several groups have undertaken meta-analyses (analyses of pooled data) of the case control studies that have been published. In 1990, Trock *et al.* [12] reported a meta-analysis of 16 case control studies and found an odds ratio for colorectal cancer of 0.57 (95% confidence interval 0.50 – 0.64) for the highest compared to the lowest quartile of fibre intake. The nature of the study did not permit discrimination between fibre types or sources.

In 1992, Howe *et al.* [13] reported a meta-analysis of 13 case control studies; the lowest odds ratio was 0.53 in the highest quintile of fibre consumption which corresponded to an intake of greater than 31 g of fibre per day. The lowest quintile consumed less than 10 g of fibre per day. The effects were consistent for both sexes and for cancers at all sites in the colon and rectum.

A further, more detailed meta-analysis by Friedenreich *et al.* [14] reached the same conclusion and showed that subjects consuming greater than 27 g of fibre per day had a 50% reduction in risk for developing colorectal cancer, compared to those consuming less than 11 g of fibre per day.

Approximately nine case control studies have examined the relationship between fibre consumption and colorectal adenomas, the pre-cursor lesion to cancer and hence a biomarker or surrogate marker for effect on cancer (see [1]). The magnitude of reduction in risk ranged from 10% to 60% in these studies but they are generally limited by small sample size and sometimes the failure to exclude the presence of adenomas in control groups.

As a general rule, studies have not attempted to differentiate between different types of fibre and fibre sources. Freudenheim *et al.* [15] made the most detailed attempt to differentiate risk for colorectal cancer with intake of cereal fibre and fruit and vegetable fibres. They found that the greatest protection, approximately 70%, was found with consumption of insoluble cereal fibre. Soluble cereal fibres were protective but to a lesser degree.

To summarise, most of the published case control studies show either a strong or moderate protective effect of dietary fibre or fibre rich foods, and thus support the fibre hypothesis. The effect is remarkably consistent and a fibre consumption of approximately 30 g per day is associated with an approximate 50% reduction in risk for developing colorectal cancer.

4.3 Prospective epidemiological studies in humans

These studies have assessed the diets of large groups of individuals and then followed them over time, sometimes with rechecking of dietary consumption. The prospective studies conducted to date have been variable in quality and design. The reader is referred to the extensive critical analysis reported by the American Gastroenterological Association [3]. Kim [3] and others [1] observed that the results were not consistent and the effect of fibre not as strong as was apparent from the case control and correlative studies. Only five of the ten studies showed a protective effect.

While there are many strengths in the prospective study design, there are several weaknesses that should be noted. The dietary analyses may not correspond to the process of cancer development. Given that the process may be as long as ten to 20 years, then studies with follow up of less than ten years may not be informative. The range of dietary intake can also be narrow within the population. One of the prospective studies [16, 24] failed to show a protective effect of consumption of dietary fibre but fibre intake only ranged up to 25 g/d. Studies where fibre intake has covered a much broader range, *e.g.*, up in excess of 35 g/d, have clearly shown an association [17]. Despite these limitations and considerations, the prospective studies, which are of generically better design, have not confirmed the strong effects seen in the case control and correlative studies.

4.4 Prospective interventional studies in humans using biomarkers

In an effort to get more direct evidence under highly controlled conditions, investigators have designed prospective intervention studies using a range of biomarkers to predict effect on oncogenesis. By the year 2000, six interventional studies – all critically analysed by Kim [3] – had been reported in humans. A Cochrane meta-analysis also addressed the available studies until 2001 [18]. No study to that time or since has used cancer as the end point due to the time factor, cost and difficulty with design and adherence to the intervention.

Biomarkers are biological events which are considered to be related to risk for development of colorectal cancer. There are several types: those that reflect a biological state considered to regulate risk, those that reflect a protective mechanism of action of an agent, and those that are events in the process of oncogenesis itself. Biomarkers are limited in their usefulness but they give insights into mechanisms of effect, and they confirm potential for agents to modify risk. Many studies over the years have addressed the effect of fibre and related dietary components on what might be termed biomarkers.

4.4.1 Studies using adenomas as an endpoint

Of the biomarkers available, adenomatous polyps are likely to be the most relevant to cancer itself. Some of the studies have been collectively reviewed [3, 18]. A trial involving patients with familial adenomatous polyposis and an ileorectal anastomosis, offered a grain fibre supplement (22.5 g/day) over a 4-year period compared to base period of 11 g/d. The intervention significantly inhibited the development of rectal polyps in those who consumed the fibre [19]. In contrast, the Toronto Polyp Prevention Trial conducted in patients who had undergone adenoma resection, found no significant differences in polyp recurrence rates between those who were counselled to follow a low-fat, high-fibre (35 g/d) diet and those consuming a typical Western diet with placebo fibre. Polyp recurrence was assessed at 2 years and results analysed on an intention to treat basis, *i.e.*, regardless of whether or not the diet was actually followed [20]. Unfortunately, there was low dietary compliance and a high drop-out rate.

The Australian Polyp Prevention Project reported that the combination of fat reduction and a supplement of wheat bran (25 g/d) reduced the incidence of large colorectal adenomas at 2 and 4 years after adenoma resection [21]. Fibre alone was not significantly protective. In the Polyp Prevention Trial [22], over 2000 people were studied for adenoma recurrence four years after resection of an adenoma. A low-fat high-fibre (18 g/d total) diet was compared to controls. The unadjusted risk ratio was 1.00 (95% confidence interval (CI), 0.90 – 1.12). This study was of sound design and pragmatic in nature. The main uncertainty was that the fibre intake was not sufficiently high to see an effect.

The Wheat Bran Fibre (WBF) trial was a double-blind, high-fibre *versus* low-fibre phase III intervention trial in which participants were randomly assigned to receive a cereal fibre supplement of either 2.0 or 13.5 g/d to assess whether a high-fibre supplement could decrease risk of recurrent colorectal adenomas [23]. Participants consumed a baseline average of 17.5 g of fibre per day, which meant that the intervention group received a substantial fibre intake (31 g/d). The multivariate adjusted odds ratio for recurrent adenoma in the high-fibre group, as compared with the low-fibre group, was 0.88 (95% CI, 0.70 – 1.11; $P = 0.28$).

The ECPO multicentre randomised trial tested the effect of diet supplementation with calcium and fibre on adenoma recurrence [24]. 665 patients with a history of colorectal adenomas were allocated to receive calcium gluconolactate and carbonate (2 g elemental calcium daily), fibre (3.5 g ispaghula husk), or placebo. Participants had colonoscopy after 3 years of follow-up. The adjusted odds ratio for recurrence was 0.66 (95% CI 0.38 – 1.17; $p = 0.16$) for calcium treatment and 1.67 (1.01 – 2.76; $p = 0.042$) for the fibre treatment. These results do not contribute as the intervention involved a low dose of a soluble fibre.

These studies did not consistently address whether the fibre consumed had influenced the colonic luminal environment. As will be seen from the animal studies below, only the types of fibre that influence the distal colonic luminal environment are protective.

Generalisability of results is limited because the end points have not involved cancer. The adenomas used as endpoints were at varying stages of progression along the oncogenic

pathway. Many of the adenomas that recurred or were studied, might not have been important in the context of colorectal cancer development [25]. These studies gave no insight into the ability of dietary fibre to inhibit or suppress progression of adenomas to cancer. It also needs to be considered that dietary fibre might be most protective when consumed in the natural food source rather than as a synthetic supplement [26].

4.4.2 Studies using other biomarkers as endpoints

The effect of dietary fibre on fermentation-related events in the colon has been extensively studied and the reader is referred to the review by Topping and Clifton [4]. A more detailed discussion of fermentation and oncogenesis is presented below.

Other biomarkers that have been studied include faecal mutagenicity [27], total and secondary faecal bile salts excretion [28, 29], and rectal epithelial proliferation in higher risk individuals [30]. All have shown effects that could be interpreted as being beneficial in the context of risk for colorectal cancer. The studies by Reddy *et al.* [27, 28] show that wheat bran (a source of insoluble fibre), but not corn or oat bran, reduces faecal concentrations of secondary bile acids such as deoxycholic acid. These biomarkers tell us about the mechanisms by which dietary fibre might protect and the stage at which they might have an impact but they do not prove an impact on cancer development.

4.5 Human studies – conclusions

After a critical review of the information available in 2000, the American Gastroenterological Association [3] concluded that on the basis of these studies it was reasonable to recommend a total dietary fibre intake of at least 35 g/d. The fibre sources should be broad and include 5 – 7 servings of a broad range of relevant foods although there is a hint that insoluble fibre is the most effective. Such consumption needed to occur in the context of general dietary guidelines as there was little evidence that fibre would be effective when the diet was otherwise unhealthy. Others have gone further and concluded that the evidence is clear and that fibre is protective [31]. Since that time, the main experimental contribution to the evidence has come from additional prospective interventional studies using adenomas as the endpoint in adenoma-prone patients. The results have not been as persuasive but the amount of fibre tested has often been well below 35 g/d and it remains to be demonstrated how informative adenomas are as a biomarker. The ideal prospective interventional study has not yet been done [32].

5 Animal studies of dietary fibre and colorectal oncogenesis

Studies in animals are useful in that they enable exploration of mechanisms of effect and impact at different points in oncogenesis. Animal models available for studying colorectal oncogenesis are exclusively rodent-based. As a general rule, animal studies that used insoluble fibres such as wheat bran consistently demonstrated protection when compared with more soluble fibres, such as oat or corn bran or pectin [31, 33 – 35]. The effect of the fibre on the luminal environment also seems important. It has been shown that active distal colonic luminal fermentation (by administering wheat bran) is more protective than fermentation in proximal colon (by administering guar gum or oat bran) [33]. The effect is also stage-specific; wheat bran exerts its protective effect at the stage in oncogenesis when dysplastic crypts begin to form [35].

These studies highlight the fact that studies in humans have not adequately addressed the impact of fibre consumption on the colonic luminal environment. As Freudenheim *et al.* [15] observed that insoluble cereal fibre was more protective than soluble cereal fibre and it has been observed that insoluble fibre has a greater impact on reduction of secondary bile salts [31], more studies are needed in humans which correlate protection with impact of fibre on the luminal environment. These models serve to test biological principles but findings need to be validated in some manner in humans before they can be considered to constitute evidence that supports public health policy. While literal translation of findings in rodents into the human setting requires some caution, they point to impact on luminal environment as being a key to protection.

6 Mechanisms of protection by dietary fibre: the issue of biological plausibility

Well before the basic nature of colorectal oncogenesis was understood, investigators postulated several mechanisms that could explain why dietary fibre might protect against development of colorectal cancer. These are summarised in Table 1. Burkitt's initial hypothesis [11] was that the mechanism was physical. Fibre increased faecal bulk, diluted carcinogens, hastened transit, and therefore reduced contact time between carcinogens and the luminal epithelium. Since then, additional mechanisms have emerged concerning fermentation, the prebiotic functions of dietary fibre, and certain general metabolic effects (Table 1).

There is evidence that points to the importance of the physical effects of dietary fibre. Certain specific binding interactions between fibre and carcinogens have been described (see [3]). In addition, fibre can directly bind bile salts [36].

Table 1. Possible mechanisms for the protective action of dietary fibre on colorectal oncogenesis

Physical
Increased bulk and dilution of carcinogen
Decreased contact time due to more rapid transit
Binding of carcinogen
Binding of bile salts
Prebiotic and metabolic action of flora
Alteration of colonic microflora; numbers and species balance
Inhibition of carcinogen activation
Stimulation of flora to increase bulk
Alteration of bile salt metabolism to reduce conversion to secondary bile salts
Fermentative
Lowering of pH
Reduced solubility of bile salts
Increased production of SCFAs, especially butyrate
Metabolic
Reduced insulin resistance and hyperinsulinaemia

As a consequence it is thought that this prevents conversion of primary to secondary bile salts and inhibits bacterial activation of pro-carcinogens.

The probiotic functions of fibre have hardly been adequately explored for their impact on colorectal oncogenesis. Fibre can exert marked changes on the colonic microflora and the luminal environment [4] bringing about changes in bacterial species and affecting production of certain microbial enzymes that are thought to be important in activation of carcinogens (*e.g.*, nitro reductase and the glycosidases) [37]. To date, however, a clear relationship between colonic microflora and the development of colorectal cancer in humans has not been demonstrated. It has been shown that dietary fibre decreases the number of anaerobic bacteria with a resultant decrease in excretion of secondary bile acids [38].

The fermentation hypothesis has initiated considerable debate. Luminal fermentation generates an acidic environment with a reduction in pH [4]. It is proposed that this reduces the potential tumour promoter activity of secondary bile acids. It also changes activity of the bacterial enzyme 7α-dehydroxylase which converts primary bile acids to secondary bile acids [39]. Acidification also increases availability of calcium to bind both bile salts and longer chain fatty acids, therefore inhibiting their effects on colonic mucosa [31]. Finally, studies in several human populations have shown that those with the lowest faecal pH have the lowest grades of colon cancer [40]. Thornton [39] hypothesised that dietary fibre through acidification of colonic contents (*via* fermentation) could protect against colon cancer. At lower pH, the enzyme 7α-dehydroxylase is inhibited (this bacterial enzyme converts primary bile acids to secondary bile acids; these secondary bile acids are considered promoters of colon cancer).

The biology becomes more complex and intriguing when considering the specific chemical products resulting from fermentation. Significant concentrations of the three principal SCFAs acetate, propionate, and butyrate are produced with total concentrations reaching 100 mM [7]. The so-called "butyrate hypothesis" is discussed in more detail below.

A unifying metabolic hypothesis has been proposed to account for all of the lifestyle factors that modulate colorectal carcinogenesis [41]. This hypothesis suggests that the lifestyle factors cause insulin resistance and hyperinsulinaemia and that these in turn stimulate growth of colorectal cancers. There are a range of *in vitro* observations involving insulin and the insulin-like growth factors that lend some support to this idea. On the other hand, risk for developing colorectal cancer increases only about 40% in diabetics [42], and the relative risk for colorectal cancer across the wide spectrum of dietary consumption is much greater than this and approximately 10-fold [43]. Therefore, this metabolic hypothesis probably only accounts for a fraction of the risk related to dietary factors.

Certainly, plausible hypotheses have been put forward to explain how dietary fibre protects against colorectal oncogenesis. But is it possible to more directly account for how such dietary factors regulate the complex events of colorectal oncogenesis? To answer this question, we must first consider what is now understood about the basis of colorectal oncogenesis.

7 The nature of colorectal oncogenesis

Colorectal cancer results from a series of genomic alterations that result in transformation of a normal epithelial cell into an adenocarcinoma [44]. The process of development of colorectal cancer is characterised by a progressively disordered genome and perturbed biology. This process is subject to external regulation and dietary lifestyle is important [1].

The molecular genesis of colorectal cancer comprises four key elements: (i) Cancer arises from a multistep process at the genomic, biological, and morphological levels. (ii) The genetic and epigenetic (*i.e.*, genomic) alterations underlying it provide a growth advantage to a clone of cells that displaces less affected cells. (iii) Genomic instability is a key characteristic of cancer and becomes progressively disordered during oncogenesis. (iv) Abnormalities occur in specific genes which are critical to maintaining the non-neoplastic phenotype. Most of the specific genetic defects that drive colorectal oncogenesis are acquired.

7.1 Precancer events in colorectal oncogenesis

Cell phenotype progressively changes during oncogenesis. The evidence suggests a broad "field" change throughout the colon characterised by cellular hyperproliferation, followed by development of focal change. The earliest focal change is the formation of cellular dysplasia within single crypts, with gradual enlargement and evolution through adenomatous polyps to carcinoma, *i.e.*, the adenoma-carcinoma sequence [45]. Two, probably inter-related models, have been proposed to describe oncogenesis at the molecular level.

7.2 The initiation-promotion model

Colorectal oncogenesis was initially described in terms of the classic "initiation-promotion model" [46, 47]. In this model, the first step involved direct damage to DNA, which resulted in mutations; *i.e.*, "initiation". Surviving mutated cells proliferated and if the mutations were biologically significant, this evolved into cancer, driven to completion by promotional factors that in themselves did not necessarily damage DNA but modified biological responsiveness. The initiation and promotion processes were conceived of as being strictly sequential as shown schematically in Fig. 1.

7.3 The multistep genomic instability model

A new model termed "multistep carcinogenesis" appears to better explain the genomic instability inherent in cancer [46, 47]. The process is largely driven by a broad range of genetic alterations, randomly accumulating in no given sequence, at multiple sites on DNA. It could be thought of as multiple, superimposed, initiation-promotion models, but such would not adequately allow for the biological complexity or widespread genomic instability characteristic of oncogenesis in the colon [46]. The critical caretaker function lost in colorectal oncogenesis is the destabilization of DNA fidelity, resulting in genomic instability [46, 47].

Genomic instability can take two main forms [44]. The first is microsatellite instability (MSI) where cells remain largely diploid. The second is chromosomal instability (CIN) where cells show aneuploidy with chromosomal breaks and other major defects in chromosomes. Cells that develop genetic abnormalities which provide a growth and/or survival advantage, may displace the neighbouring normal cells through successive waves of cellular clonal expansion and selection. With time, a subclone may acquire the full malignant phenotype. Eventually, further biological changes may occur which confer metastatic capacities. The relationship between these two models is shown diagrammatically in Fig. 1.

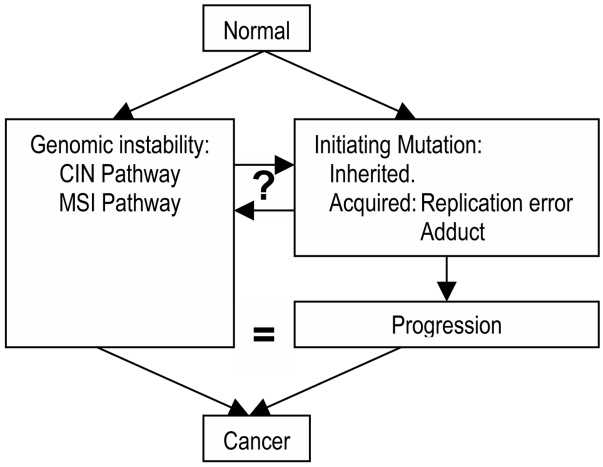

Figure 1. Diagrammatic representation of the two main models of colorectal oncogenesis. On the right is the initiation-promotion pathway and on the left the multistep genomic instability pathway. The concept of progression is consistent with, and essentially equal to, progressive genomic instability. Mutations, whether inherited or acquired, can clearly contribute to or result from genomic instability. Hence, these two models appear to be inter-related. CIN, chromosomal instability; MSI, microsatellite instability.

7.4 Causes of genomic instability

Gene dysfunction is the driving force behind oncogenesis [44, 46, 47]. The trigger might be inherited as is the case in hereditary nonpolyposis colorectal cancer (HNPCC) where a mutation in a DNA mismatch repair gene is passed on, or in familial adenomatous polyposis (FAP) where a mutation in the *APC* gene is inherited. Acquired sources of genetic abnormality may arise as a result of chance mutations occurring as part of the normal cellular lifespan, or to lifestyle, or to exposure to dietary carcinogens, or they may be less targeted and arise as a result of progressive genomic instability [44].

Several environmental factors predictably induce damage to DNA, including viral infections, chemical carcinogens, and radiation (ionising radiation, such as X-rays and γ-rays, and particle radiation, such as electrons, α particles, and heavy ions). With colorectal cancer, viral infections and radiation are rare causes but dietary chemical carcinogens are important because of the direct exposure of the gut.

7.5 Chemical carcinogenesis

Chemical carcinogens are ubiquitous in the human diet and studies have demonstrated increased mutagenic activity in the stools of patients at risk for colorectal neoplasia [48]. Typically, carcinogens occur in foods in a form needing to

be activated (*i. e.*, procarcinogen) and are modified by metabolic processes in liver, colonic lumen, and colonic mucosa to either activate or deactivate them. The microbial flora of the gastrointestinal tract and certain phytochemicals (bioactive components of plants) are also important in their activation and deactivation [49].

Point mutation, *i. e.*, a change in a single base pair, affects a range of genes important in human colorectal oncogenesis. These include the oncogene *K-RAS*, and the tumour suppressor gene *p53*. O^6-methylguanine (O^6-MeG) adducts are increased in the colons of cancer prone regions in humans [50] implicating alkylating agents in pathogenesis. GC→AT transitional mutations (characteristic of O^6-MeG adducts) account for the majority of mutations found in K-*ras* in human CRC [51] and are also common in p53 [52].

Point mutations can arise through a variety of spontaneous and induced mechanisms. Mutations may be spontaneous due to the instability of the purine and pyrimidine bases themselves. If these are not corrected by the inherent surveillance and repair systems (DNA checkpoint genes, MMR genes) they are perpetuated during the next round of replication as a mutation ([53], also see [44]). Carcinogens may cause a mutation by first creating a DNA adduct. While the type of adduct depends on the chemistry of the mutagen, a major proportion are due to alkylating or oxidative agents. These agents respectively cause alkyl groups or reactive oxygen species (ROS) to covalently bond to DNA bases to create the adduct [54]. O^6-MeG [55] and 8-hydroxy 2′-deoxyguanosine (8-OH-dG) [51] are the most frequent adducts induced by alkylating agents and ROS, respectively. If unrepaired, an adduct might lead to pathogenetically important mutations.

7.6 Cellular response to genomic damage

If damage occurs to DNA, and provided that cellular recognition and surveillance systems detect this, the cell responds in two main ways. One is cell cycle arrest to allow DNA repair [56] through enzymes, such as MGMT. The other involves activation of apoptosis if it cannot be repaired [57]. If both fail, further checkpoint repair systems may come in to play when the cell attempts to proliferate (S-phase). This is shown diagrammatically in Fig. 2.

The acute apoptotic response to genotoxin-induced damage (AARGC) might be the most important homeostatic control mechanism [58]. It occurs at least as rapidly as activation of DNA repair mechanisms and occurs exclusively in the proliferative compartments of the crypt where it can be shown that DNA adducts occur (Fig. 3) [57, 58]. If DNA adducts persist because they escape cellular repair mechanisms and apoptotic deletion, they may lead to the fixation of a pathogenic mutation. If a permanent mutation occurs in a critical region of an oncogene or tumour suppress gene, it can lead to activation of the oncogene or deactivation of tumour suppress gene. These multiple changes will lead to aberrant cells with loss of normal growth control and genetic fidelity, and ultimately to cancer [59, 60].

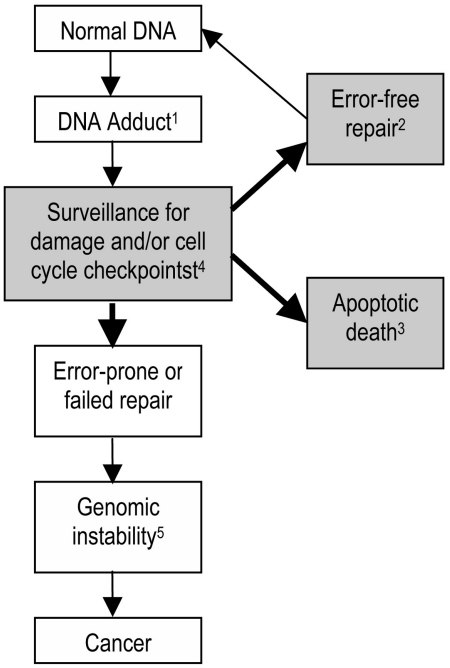

Figure 2. Diagrammatic representation of a model that could account for control of mutations contributing to colorectal oncogenesis. The three shaded boxes represent key events in the process that act to control the consequences of DNA adduct formation. The three heavy arrows indicate the major outcomes of inherent surveillance mechanisms for controlling DNA fidelity in response to adduct formation. Failed repair results in adduct "fixation" as a mutation that is passed on to cell progeny. Genomic instability can itself compromise all control mechanisms. The numbered superscripts represent points subject to environmental regulation by a variety of mechanisms. Epigenetic regulation can apply at all of these.

8 How do dietary regulators affect genomic instability and/or mutations?

If procarcinogens in the diet do drive colorectal oncogenesis, then dietary manipulation may well be possible either by decreasing exposure, minimising damage or activating repair. The molecular biological methods to study this are only now becoming available.

Dietary factors might also act to counteract the biological consequences of genomic instability. Activation of apoptosis is one obvious mechanism as it removes genetically disordered cells. Reduction in S-phase and cell proliferation is

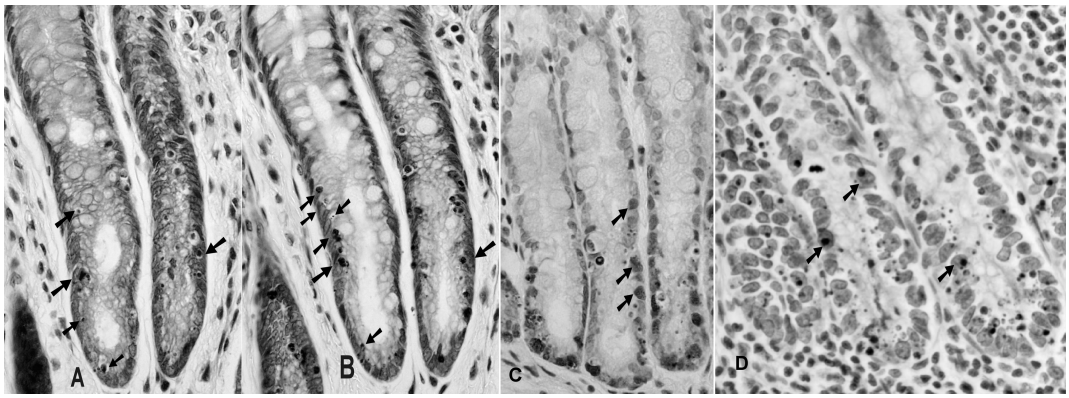

Figure 3. Response in rat colonic epithelium to injection of the methylating carcinogen azoxymethane (8 h after injection). (A) AARGC with typical apoptotic cells indicated by arrows (H&E stain). (B) AARGC with typical apoptotic cells indicated by arrows (TUNEL stain). (C) Immunohistochemical localisation of cells in crypt in S-phase by staining with PCNA antibody. Typical stained cells shown by arrows. (D) Immunohistochemical localisation of cells possessing methyl-DNA adducts. Anti O^6-MeG antibody used was kindly provided by Prof. Jürgen Thomale, Institute of Cell Biology, West German Cancer Centre, Essen. Typical stained cells which are also apoptotic are shown by arrows.

another as it reduces exposure of DNA to carcinogens and facilitates repair. Proving that these are the operative mechanisms is challenging.

Factors might also regulate expression of key genes either directly or indirectly. Again, the evidence is suggestive for a few agents but has not been systematically explored, largely because the genetic factors driving oncogenesis are complex and oncogenesis is not driven by any single genetic process.

9 Fermentation, oncogenesis, and butyrate

Evidence is now accumulating to support a modulatory effect on oncogenesis of colonic fermentation. The evidence that NSPs protect *via* the results and/or products of fermentation has been reviewed (see [61]) and some of the evidence has been discussed above. The SCFA butyrate has generated the most interest [62, 63] and it may be a protective factor against colorectal cancer [61, 63]. Although butyrate is the primary energy source for colonic epithelium [64], it inhibits growth of cancer cells *in vitro* and forces a more normal differentiated phenotype [62]. In addition, it is a potent pro-apoptotic agent [65] which might aid removal of cells with damaged DNA. Colonic production of butyrate by fermentation is associated with reduced tumour mass in an animal model, provided that fermentation is active in distal colon [33]. Such *in vivo* evidence is not consistent in animal models however as differently designed studies do not always reach the same conclusion [66].

The evidence that fermentation is important to protection in humans remains indirect as there have been insufficient well-designed studies which address the epidemiology of faecal fermentation events in relation to risk for colorectal cancer. In a small cohort study, faeces from patients with colorectal neoplasia produced less butyrate than faeces from controls [67]. Walker *et al.* [40] found in several African populations that the lowest pH coincided with the lowest incidence of colorectal cancer. This appears to be the only obvious attempt to study fermentation-dependent effects in the colonic luminal environment at the population level.

Several groups have pursued the biological effect of butyrate in view of its potential to link fermentation with control of molecular events characterising oncogenesis. Whitehead *et al.* [62] showed that butyrate alone of the principal SCFAs produced by fermentation, induced a differentiated phenotype in cancer cells and slowed their proliferation. Subsequent studies, discussed below, have demonstrated the capability of butyrate to regulate gene expression in a manner that might be relevant to oncogenesis.

9.1 Butyrate and the cell cycle

At the cell biology level, butyrate in concentrations of just a few mM inhibits proliferation, induces differentiation and induces apoptosis. It induces $p21^{WAF1/Cip1}$ at the protein and mRNA levels [68 – 70] which brings about a block in the cell cycle at G1 and so inhibits cell proliferation. Exactly how butyrate regulates p21 is unclear. By blocking the cell cycle at G1, it might allow DNA checkpoint-mediated repair of genomic instability or mutations. In comparing the effects of butyrate and trichostatin on gene expression in a colorectal cancer cell line, it was found that 23 genes were modulated by both inhibitors of histone deacetylase [71]. A

major gene affected is *tob-1,* a DNA checkpoint gene involved in the cell cycle.

9.2 Butyrate and apoptosis: an epigenetic effect

In parallel with its effects on the cell cycle, butyrate also induces apoptosis. Butyrate is able to induce apoptosis in various cell lines [65, 72, 73]. Recent evidence suggests that butyrate induces apoptosis through a histone hyperacetylation–mediated pathway, which results in the conversion of caspase-3 from its proenzyme form to the catalytically active protease [65]. By inhibiting histone deacetylase, it results in relative hyperacetylation of core histone proteins (H3 and H4) [74]. Hyperacetylation of histones disrupts ionic interactions with the adjacent DNA backbone, creating less densely packed chromatin, or euchromatin, and allowing transcription factors to activate specific genes [75].

Epigenetic events are defined as alterations in gene expression without changes in the DNA coding sequences that are heritable through cell division. Epigenetic changes generally occur during the early phases of cancer development [76]. There is evidence that certain epigenetic changes may be reversed by the use of molecules with the ability to inhibit histone deacetylase [76]. Thus, butyrate may play an important role through inhibition of histone deacetylase in reversing epigenetic events inherent in oncogenesis as well as in activating apoptosis.

This epigenetic action of butyrate seems likely to occur *in vivo* because rats fed a high-fibre diet had high luminal butyrate levels [5, 33], and this was associated with histone hyperacetylation and growth inhibition in colonic epithelial cells [77]. In addition, Le Leu *et al.* [78] found that luminal butyrate concentrations were correlated with the acute apoptotic response to a genotoxic carcinogen in the colonic epithelium of rats *in vivo*.

9.3 Butyrate and cellular differentiation

Butyrate is able to induce differentiation of neoplastic colonocytes *in vitro* producing a phenotype typically associated with the normal mature cell [62]. In efforts to explain the molecular basis of this effect, various studies using differential display have demonstrated that several hours after adding butyrate to cells in culture, expression of many genes changes [79]. Which are the keys to this effect are unclear. Some may be affected by the epigenetic mechanism related to the effect on histone deacetylase. But others might be due to a direct effect of butyrate on response elements in gene regulatory regions [80]. BRF1 (butyrate response factor 1) is a member of an immediate early gene family specifying putative nuclear transcription factors [81]. More recently, BRF1 has been shown to be an essential regulator of AU-rich element-dependent mRNA turnover [82]. Butyrate is able to repress BRF1 transcription in human colorectal cancer cell lines [83].

9.4 Butyrate transport in cancer cells

Over a decade ago, it was observed that butyrate had different effects on cancer compared to normal cells [84]. This might be a result of transmembrane transport of butyrate becoming abnormal in cancer cells. The transport of butyrate across the luminal membrane of the colonic epithelium is predominantly mediated by a monocarboxylate transporter (MCT1) [85]. MCT1 protein expression appears to be significantly greater in healthy colonic tissue compared to neoplastic colonic tissue [86]. While the molecular mechanisms involved in the downregulation of MCT1 expression are largely unknown, a possible mechanism may be due to reduced substrate availability that is an inadequate supply of dietary fibre or resistant starch [87].

10 Regulation of genotoxin-induced apoptosis *in vivo*

As outlined above, there is an AARGC in the colon [57, 58]. AARGC is dependent on *p53* [88] and when defective, leads to increased genomic instability and greater risk for colorectal cancer [88]. A recent study has shown that feeding fermentable wheat bran to rats increases AARGC while feeding nonfermentable methylcellulose does not (Fig. 4) [58]. Feeding type-2 resistant starch (as high amylose maize starch) also enhances AARGC; furthermore, butyrate levels in the faeces have been shown to correlate significantly with AARGC in distal colonic crypts suggesting that butyrate is the mediator of this effect [78]. These studies provide

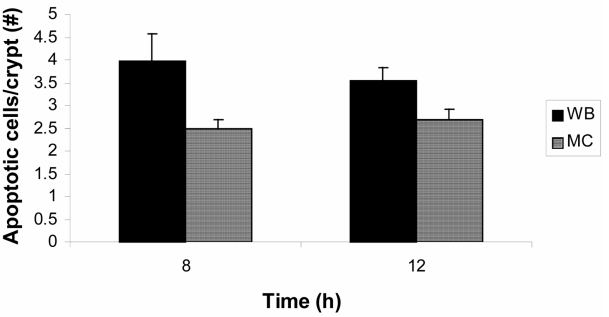

Figure 4. Effects of fermentable fibre wheat bran and nonfermentable fibre methylcellulose on genotoxin-induced apoptosis in response to azoxymethane (AOM), in distal colon, at 8 and 12 h after administration of AOM [58]. The difference in effects of fibre on distal colon was significant ($p < 0.01$). Vertical bars represent SEM.

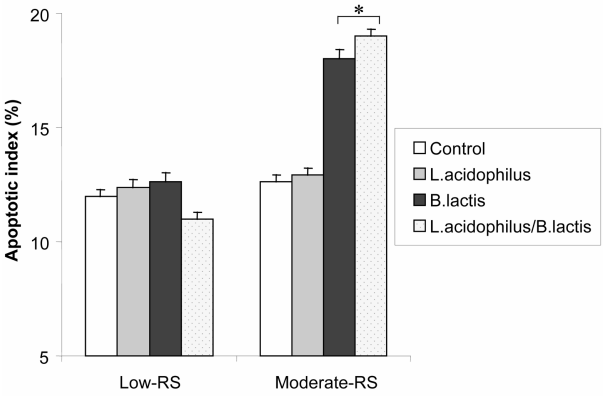

Figure 5. Apoptotic index (8 h after administration of azoxymethane) in the distal colon of RATS fed a low-resistant starch or moderate-resistant starch diet supplemented with *Lactobacillus acidophilus* and/or *Bifidobacterium lactis* [89]. Values are means ± SEM, $n = 12$. * $P < 0.001$ compared with all other treatment groups.

evidence that the products of fermentation can act to enhance homeostatic mechanisms for controlling mutational load and maintaining genomic stability. Of course, aspects of fermentation other than butyrate may contribute.

More recent work from the same laboratory has examined the interaction between fermentable substrate (prebiotic) and probiotic bacteria. The question posed was whether the synbiotic combination of resistant starch with B lactis was capable of regulating AARGC [89]. As can be seen in Fig. 5, low doses of resistant starch or B lactis alone had no effect on AARGC, but a synbiotic combination of the same agents did enhance AARGC. Studies are now underway to formally test if this reduces mutational load, genomic instability and risk for colorectal cancer in animal models.

11 Conclusions

The diverse nature of dietary fibre and its resultant fate in the gut makes it difficult to generalise about its value in relation to colorectal oncogenesis. The evidence indicates that different types of fibre create different conditions in different regions of the gut. This is reflected in different effects on oncogenesis, especially in animal models but perhaps also in humans. Data from animal models show that insoluble fibre is protective. Evidence from human studies are not consistent, especially considering the interventional studies. However, all such studies have been dependent on measuring the effect of an intervention on biomarkers short of cancer formation. Ability to slow progression from adenoma to cancer has not been studied. Furthermore, the interventions tested have not always been shown to impact upon the colonic luminal environment in a manner that might be protective. The biological and molecular events characteristic of colorectal oncogenesis, while complex, provide some insight into how fibre ingestion might regulate oncogenesis. While several mechanisms might account for protection, the results of fermentation and especially butyrate production provide examples of how genomic instability might be controlled. Butyrate has effects on biology that might control genomic instability or minimise mutational load. It activates apoptosis and blocks the cell cycle, allowing DNA checkpoint surveillance of the genome and perhaps correction of genomic events that drive oncogenesis. Butyrate itself can regulate gene expression by both epigenetic and direct effects. More work is now needed to more precisely determine the conditions under which fibre exerts a protective effect. Once we understand this, we will be in a better position to design the studies that clarify exactly what should be done with the diet to aid prevention of colorectal cancer. In the meantime it seems prudent to consume more than 30 g of fibre per day and to include a food source of insoluble fibre.

12 References

[1] World Cancer Research Fund, Cancers: Colon, Rectum, in: *Food, Nutrition and the Prevention of Cancer: a Global Perspective.* American Institute for Cancer Research, Washington 1997, pp. 216 – 251.

[2] Ferguson, L. R., Chavan, R. R., Harris, P. J., Changing concepts of dietary fiber: implications for carcinogenesis. *Nutr. Cancer* 2001, *39*, 155 – 169.

[3] Kim, Y. I., AGA Technical Review: Impact of dietary fiber on colon cancer occurrence. *Gastroenterology* 2000, *118*, 1235 – 1257.

[4] Topping, D. L., Clifton, P. M., Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol Rev.* 2001, *81*, 1031 – 1064.

[5] McIntyre, A., Young, G. P., Taranto, T., Gibson, P. R., Ward, P., Different fibers have different regional effects on luminal contents of rat colon. *Gastroenterology* 1991, *101*, 1274 – 1281.

[6] Folino, M., McIntyre, A., Young, G. P., Dietary fibers differ in their effects on large bowel epithelial proliferation and fecal parameters of fermentation in the rat. *J. Nutrition* 1995, *125*, 1521 – 1528.

[7] Cummings, J. H., Macfarlane, G. T., The control and consequences of bacterial fermentation in the human colon: a review. *J. Appl. Bacteriol*. 1991, *70*, 443 – 459.

[8] Macfarlane, G. T., Macfarlane, S., Human colonic microbiota: ecology, physiology and metabolic potential of intestinal bacteria. *Scand. J. Gastroenterol*. 1997, *222 (Suppl.)*, 3 – 9.

[9] Cummings, J. H., Pomare, E. W., Branch, W. J., Naylor, C. P., MacFarlane, G. T., Short-chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* 1987, *28*, 1221 – 1227.

[10] Cummings, J. H., Beatty, E. R., Kingman, S. M., Bingham, S. A., Englyst, H. N., Digestion and physical properties of resistant starch in the human large bowel. *Br. J. Nutr.* 1996, *75*, 733 – 747.

[11] Burkitt, D. P., Epidemiology of cancer of the colon and rectum. *Cancer* 1971, *28*, 3 – 13.

[12] Trock, B., Lanza, E., Greenwald, P., Dietary fiber, vegetables, and colon cancer: Critical review and meta-analyses of the epidemiologic evidence. *J. Natl. Cancer Inst.* 1990, *82*, 650 – 661.

[13] Howe, G. R., Benito, E., Castelleto, R., Cornee, J., *et al*., Dietary intake of fiber and decreased risk of cancers of the colon and rectum: evidence from the combined analysis of 13 case-control studies. *J. Natl. Cancer Inst.* 1992, *84*, 1887 – 1896.

[14] Friedenreich, C. M., Brant, R. F., Riboli, E., Influence of methodological factors in a pooled analysis of 13 case-control studies of colorectal cancer and dietary fiber. *Epidemiology* 1994, *5*, 66 – 79.

[15] Freudenheim, J. L., Graham, S., Horvath, P. J., Marshall, J. R., *et al*., Risks associated with source of fiber and fiber components in cancer of the colon and rectum. *Cancer Res.* 1990, *50*, 3295 – 3300.

[16] Fuchs, C. S., Giovannucci, E. L., Colditz, G. A., Hunter, D. J., *et al*., Dietary fiber and the risk of colorectal cancer and adenoma in women. *N. Engl. J. Med.* 1999, *340*, 169 – 176.

[17] Reddy, B. S., Sharma, C., Simi, B., Engle, A., *et al*., Metabolic epidemiology of colon cancer: effect of dietary fiber on fecal mutagens and bile acids in healthy subjects. *Cancer Res.* 1987, *47*, 644 – 648.

[18] Asano, T., McLeod, R. S., Dietary fibre for the prevention of colorectal adenomas and carcinomas. *Cochrane Database Syst. Rev.* 2002, CD003430.

[19] DeCosse, J. J., Miller, H. H., Lesser, M. L., Effect of wheat fiber and vitamins C and E on rectal polyps in patients with familial adenomatous polyposis. *JNCI* 1989, *81*, 1290 – 1297.

[20] McKeown, McKeown-Eyssen, G. E., Bright-See, E., Bruce, W. R., Jazmaji, V., *et al*., A randomized trial of a low fat high fibre diet in the recurrence of colorectal polyps. Toronto Polyp Prevention Group. *J. Clin. Epidemiol.* 1994, *47*, 525 – 536.

[21] Maclennan, R., Macrae, F., Bain, C., Battistutta, D., *et al*., Randomized trial of intake of fat, fiber and beta carotene to prevent colorectal adenomas. *JNCI* 1995, *87*, 1760 – 1766.

[22] Schatzkin, A., Lanza, E., Corle, D., Lance, P., *et al*., Lack of effect of a low-fat, high-fiber diet on the recurrence of colorectal adenomas. *N. Engl. J. Med.* 2000, *342*, 1149 – 1155.

[23] Alberts, D. S., Martinez, M. E., Roe, D. J., Guillen-Rodriguez, *et al*., Lack of effect of a high-fiber cereal supplement on the recurrence of colorectal adenomas. *N. Engl. J. Med.* 2000, *342,* 1156 – 1162.

[24] Bonithon-Kopp, C., Kronborg, O., Giacosa, A., Rath, U., Faivre, J., Calcium and fibre supplementation in prevention of colorectal adenoma recurrence: a randomised intervention trial. *Lancet* 2000, *356*, 1300 – 1306.

[25] Atkin, W. S., Morson, B. C., Cuzick, J., Long-term risk of colorectal cancer after excision of rectosigmoid adenomas. *N. Engl. J. Med.* 1992, *326*, 658 – 662.

[26] Ferguson, L. R., Harris, P. J., Protection against cancer by wheat bran: role of dietary fibre and phytochemicals. *Eur. J. Cancer Prev.* 1999, *8*, 17 – 25.

[27] Reddy, B., Engle, A., Katsifis, S., Simi, B., *et al*., Biochemical epidemiology of colon cancer: effect of types of dietary fiber on fecal mutagens, acid, and neutral sterols in healthy subjects. *Cancer Res.* 1989, *49,* 4629 – 4635.

[28] Reddy, B. S., in: Micozzi, M. S., Moon, T. E. (Eds.), Animal experimental evidence on macronutrients and cancer in macronutrients: Investigating their role in cancer, Marcel Dekker, New York 1992, pp. 33 – 54.

[29] Alberts, D. S., Ritenbaugh, C., Story, J. A., Aickin, M., *et al*., Randomised, double-blinded, placebo-controlled study of effect of wheat bran fiber and calcium on fecal bile acids in patients with resected adenomatous colon polyps. *JNCI* 1996, *88*, 81 – 92.

[30] Alberts, D. S., Einspahr, J., Rees McGee, S., Ramanujam, P., *et al*., Effects of dietary wheat bran fiber on rectal epithelial cell proliferation in patients with resection for colorectal cancers. *JNCI* 1990, *82*, 1280 – 1285.

[31] Reddy, B. S., The Fourth DeWitt, S. Goodman lecture. Novel approaches to the prevention of colon cancer by nutritional manipulation and chemoprevention. *Cancer Epidemiol. Biomarkers Prev.* 2000, *9*, 239 – 247.

[32] Hill, M., Dietary fibre and colon cancer: where do we go from here? *Proc. Nutr. Soc.* 2003, *62*, 63 – 65.

[33] McIntyre, A., Gibson, P. R., Young, G. P., Butyrate production from dietary fibre and protection against large bowel cancer in a rat model. *Gut* 1993, *34*, 386 – 391.

[34] Jacobs, L. R., Effect of dietary fiber on colonic cell proliferation and its relationship to colon carcinogenesis. *Prev. Med.* 1987, *16*, 566 – 571.

[35] Young, G. P., McIntyre, A., Albert, V., Folino, M., *et al*., Wheat bran suppresses potato starch-potentiated colorectal tumorigenesis at the aberrant crypt stage in a rat model. *Gastroenterology* 1996, *110*, 508 – 514.

[36] Story, J. A., Kirtchevsky, D., Comparison of the binding of various bile acids and bile salts *in vitro* by several types of fiber. *J. Nutr.* 1976, *106*, 1292 – 1294.

[37] Goldin, B. R., Gorbach, S. L., The relationship between diet and rat fecal bacterial enzymes implicated in colon cancer. *JNCI* 1976, *57*, 371 – 375.

[38] Jacobs, L. R., Fiber and colon cancer. *Gastroenterol. Clin. North Am.* 1988, *17*, 747 – 759.

[39] Thorton, J. R., High colonic pH promotes colorectal cancer. *Lancet* 1981, *1*, 1081 – 1082.

[40] Walker, A. R. P., Walker, B. F., Walker, A. J., Faecal pH, dietary fiber intake and proneness to colon cancer in four South African populations. *Br. J. Cancer* 1986, *53*, 489 – 495.

[41] Giovannucci, E., Insulin and colon cancer. *Cancer Causes Control* 1995, *6*, 164 – 179.

[42] Weiderpas, E., Gridley, G., Nyren, O., Ekbom, A., *et al*., Diabetes mellitus and risk of large bowel cancer. *J. Natl. Cancer Inst.* 1997, *89*, 660 – 661.

[43] Kune, S., Kune, G. M., Watson, F., Case-control study of dietary etiologic factors. The Melbourne colorectal cancer study. *Nutr. Cancer* 1987, *9*, 21 – 42.

[44] Grady, W. M., Genomic instability and colon cancer. *Cancer Metast. Rev.* 2004, *23*, 11 – 27.

[45] Morson, B. C., Evolution of cancer of colon and rectum. *Cancer* 1974, *34*, 845 – 849.

[46] Boland, C. R., Malignant tumors of the colon. In: Yamada, T, Alpers, D. H., Kaplowitz, N., Laine, L., Owyang, C., Powell, D.W. (Eds.), *Textbook of Gastroenterology*, Lippincott, Williams and Wilkins, Philadelphia, PA 2003, pp. 1940 – 1989.

[47] Carethers, J. M., Boland, C. R., Neoplasia of the gastrointestinal tract. In: Yamada, T., Alpers, D. H., Kaplowitz, N., Laine, L., Owyang, C., Powell, D.W. (Eds.), *Textbook of Gastroenterology*, Lippincott, Williams and Wilkins, Philadelphia, PA 2003, pp. 557 – 583.

[48] Lang, N. P., Butler, M. A., Massergill, J., *et al.*, Rapid metabolic phenotypes for acetyltransferase and cytochrome p4501A2 and putative exposure to food-borne heterocyclic amines increase the risk for colorectal cancer or polyp. *Cancer Epidemiol. Biomarkers Prev.* 1994, *3*, 675 – 682.

[49] Miller, E. C., Miller, J. A., Searches for ultimate chemical carcinogens and their reactions with cellular macromolecules. *Cancer* 1981, *47*, 2327 – 2345.

[50] Povey, A. C., *et al*., Elevated levels of the pro-carcinogenic adduct, O^6-methylguanine, in normal DNA from the cancer prone regions of the large bowel. *Gut* 2000, *47*, 362 – 365.

[51] Bos, J. L., Ras oncogenes in human cancer: a review. *Cancer Res.* 1989, *49*, 4682 – 4689.

[52] Hollstein, M., *et al*., Mutations in human cancers. *Science* 1991, *253*, 49 – 53.

[53] Lengauer, C., Kinzler, K. W., Vogelstein, B., Genetic instability in colorectal cancers. *Nature* 1997, *386*, 623 – 627.

[54] Hochstein, P., Atallah, A. S., The nature of oxidants and antioxidant systems in the inhibition of mutation and cancer. *Mutat. Res.* 1988, *202*, 363 – 375.

[55] Povey, A. C., Badawi, A. F., Cooper, D. P., Hall, C. N., *et al*., DNA alkylation and repair in the large bowel: animal and human studies. *J. Nutr.* 2002, *132*, 3518S – 3521S.

[56] Lane, D. P., p53, guardian of the genome. *Nature Cancer* 1992, *358*, 15 – 16.

[57] Hong, M. Y., Chapkin, R. S., Wild, C. P., Morris, J. S., *et al*., Relationship between DNA adduct levels, repair enzyme, and apoptosis as a function of DNA methylation by azoxymethane. *Cell Growth Differ.* 1999, *10*, 749 – 758.

[58] Hu, Y., Martin, J., Le Leu, R., Young, G. P., The colonic response to genotoxic carcinogens in the rat: regulation by dietary fibre. *Carcinogenesis* 2002, *23*, 1131 – 1137.

[59] Potten, C. S., Grant, H. K., The relationship between ionizing radiation-induced apoptosis and stem cells in the small and large intestine. *Br. J. Cancer* 1998, *78*, 993 – 1003.

[60] Renehan, A. G., Bach, S. P., Potten, C. S., The relevance of apoptosis for cellular homeostasis and tumorigenesis in the intestine. *Can. J. Gastroenterol.* 2001, *15*, 166 – 176.

[61] Cassidy, A., Bingham, S. A., Cummings, J. H., *Br. J. Cancer* 1994, *69*, 937 – 942.

[62] Whitehead, R. H., Young, G. P., Bhathal, P. S., Effects of SCFA on a new human colon carcinoma cell line (LIM1215). *Gut* 1987, *27*, 1457 – 1463.

[63] Stephen, A. M., Cumming, J. H., Mechanism of action of dietary fibre in the human colon. *Nature* 1980, *284*, 283 – 284.

[64] Roediger, W. E., Utilization of nutrients by isolated epithelial cells of the rat colon. *Gastroenterology* 1982, *83*, 424 – 429.

[65] Medina, V., Edmonds, B., Young, G. P., James, R., *et al*., Induction of caspase-3 protease activity and apoptosis by butyrate and trichostatin A (inhibitors of histone deacetylase): dependence on protein synthesis and synergy with a mitochondrial/cytochrome c-dependent pathway. *Cancer Res.* 1997, *57*, 3697 – 3707.

[66] Zoran, D. L., Turner, N. D., Taddeo, S. S., Chapkin, R. S., *et al*., Wheat bran diet reduces tumor incidence in a rat model of colon cancer independent of effects on distal luminal butyrate concentrations. *J. Nutr.* 1997, *127*, 2217 – 2225.

[67] Clausen, M. R., Bonnen, H., Mortensen, P. B., Colonic fermentation of dietary fibre to short chain fatty acids in patients with adenomatous polyps and colonic cancer. *Gut* 1991, *32*, 923 – 928.

[68] Archer, S. Y., Meng, S., *et al*., "p21(WAF1) is required for butyrate-mediated growth inhibition of human colon cancer cells." *Proc. Natl. Acad. Sci. USA* 1998, *95*, 6791 – 6797.

[69] Siavoshian, S., Segain, J. P., *et al*., "Butyrate and trichostatin A effects on the proliferation/differentiation of human intestinal epithelial cells: induction of cyclin D3 and p21 expression." *Gut* 2000, *46*, 507 – 514.

[70] Chai, F., Evdokiou, A., Young, G. P., Zalewski, P. D., Involvement of $p21^{Waf1/Cip1}$ and its cleavage by DEVD-caspase in apoptosis of colorectal cancer cells by butyrate. *Carcinogenesis* 2000, *21*, 7 – 14.

[71] Della Ragione, F., Criniti, V., Della Pietra, V., Borriello, A., *et al*., Genes modulated by histone acetylation as new effectors of butyrate activity. *FEBS Lett.* 2001, *499*, 199 – 204.

[72] Hague, A., Elder, D. J., Hicks, D. J., Paraskeva, C., Apoptosis in colorectal tumour cells: induction by the short chain fatty acids butyrate, propionate and acetate and by the bile salt deoxycholate. *Int. J. Cancer* 1995, *60*, 400 – 406.

[73] Heerdt, B. G, Houston, M. A., Augenlicht, L. H., Short-chain fatty acid-initiated cell cycle arrest and apoptosis of colonic epithelial cells is linked to mitochondrial function. *Cell Growth Differ.* 1997, *8*, 523 – 532.

[74] Sealy, L., Chalkley, R., The effect of sodium butyrate on histone modification. *Cell* 1978, *14*, 115 – 121.

[75] Grunstein, M., Histone acetylation in chromatin structure and transcription. *Nature (Lond.)* 1997, *389*, 349 – 352.

[76] Kopelovich, L., Crowell, J. A., *et al*., The epigenome as a target for cancer chemoprevention. *JNCI* 2003, *95*, 1747 – 1757.

[77] Boffa, L. C., Lupton, J. R., Mariani, M. R., Modulation of colonic epithelial cell proliferation, histone acetylation, and luminal short chain fatty acids by variation of dietary fiber in rats. *Cancer Res.* 1992, *52*, 5906 – 5912.

[78] Le Leu, R. K., Hu, Y., Young, G. P., Effects of resistant starch and nonstarch polysaccharides on colonic lumenal environment and genotoxin-induced apoptosis in the rat. *Carcinogenesis* 2002, *23*, 713 – 719.

[79] Williams, E. A., Coxhead, J. M., Mathers, J. C., Anti-cancer effects of butyrate: use of micro-array technology to investigate mechanisms. *Proc. Nutr. Soc.* 2003, *62*, 107 – 115.

[80] Kruh, J., Tichonicky, L., Defer, N., Effect of butyrate on gene expression. In: Binder, H. J., Cummings, J. H., Soergel, K. (Eds.), *Short-chain fatty acids*, Kluwer, Dordrecht, The Netherlands 1994, pp. 135 – 147.

[81] Maclean, K. N., See, C. G., McKay, I. A., Bustin, S. A., The human immediate early gene BRF1 maps to chromosome 14q22-q24. *Genomics* 1995, *30*, 89 – 90.

[82] Stoecklin, G., Colombi, M., *et al*., Functional cloning of BRF1, a regulator of ARE-dependent mRNA turnover. *Embo J.* 2002, *21*, 4709 – 4718.

[83] Maclean, K. N., McKay, I. A., *et al*., Differential effects of sodium butyrate on the transcription of the human TIS11 family of early-response genes in colorectal cancer cells. *Br. J. Biomed. Sci.* 1998, *55*, 184 – 191.

[84] Young, G. P., Gibson, P. R., Contrasting effects of butyrate on proliferation and differentiation of normal and neoplastic cells. In: Rombeau, J. L., Cummings, J. H., Sakata, T. (Eds.), *Short-chain fatty acids: Metabolism and Clinical Importance*, Ross Laboratories, Columbus, OH 1991, pp. 50 – 55.

[85] Ritzhaupt, A., Ellis, A., Hosie, K. B., Shirazi-Beechey, S. P., Identification and characterization of a monocarboxylate transporter (MCT1) in pig and human colon: its potential to transport L-lactate as well as butyrate. *J. Physiol. (Lond.)* 1998, *507*, 819 – 830.

[86] Lambert, D. W., Wood, I. S., Ellis, A., Shirazi-Beechey, S. P., Molecular changes in the expression of human colonic nutrient transporters during the transition from normality to malignancy. *Brit. J. Cancer* 2002, *86*, 1262 – 1269.

[87] Cuff, M. A., Lambert, D. W., Shirazi-Beechey, S. P., Substrate-induced regulation of the human colonic monocarboxylate transporter, MCT1. *J. Physiol.* 2002, *539*, 361 – 371.

[88] Hu, Y., Le Leu, R. K., Young, G. P., Absence of acute apoptotic response to genotoxic carcinogens ("AARGC") in p53 deficient mice is associated with increased susceptibility to azoxymethane-induced colon tumours. *Int. J. Cancer* 2005, in press.

[89] Le Leu, R. K., Brown, I. L., Hu, Y., Bird, A. R., *et al*., Synbiotic combination of resistant starch and bifidobacterium lactis can facilitate apoptotic deletion of carcinogen-damaged cells in the rat colon. *J. Nutr.* 2005, in press.

