We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

9

The Molecular Genetic Events in Colorectal Cancer and Diet

Adam Naguib[1], Laura J Gay[2], Panagiota N Mitrou[3] and Mark J Arends[4]
[1]*Cold Spring Harbor Laboratory*
[2]*Queen Mary, University of London*
[3]*Medical Research Council Centre for Nutritional Epidemiology in Cancer Prevention and Survival*
[4]*Department of Pathology, Addenbrooke's Hospital, University of Cambridge*
[1]*USA*
[2,3,4]*UK*

1. Introduction

Compelling evidence suggests that dietary intakes directly influence colorectal cancer (CRC) risk. Initial observations that CRC incidence is not ubiquitous worldwide, with incidence rates varying up to twenty-five fold between populations (Parkin et al., 2005), indicate the large degree to which this cancer type is influenced by diet and environment. Additionally, observations that migration of individuals confers rapid (within one generation) adoption of the CRC incidence of the host population (Boyle & Langman, 2000; McMichael & Giles, 1988), suggest that dietary and environmental factors determine the risk of colorectal neoplasia to a degree similar to, or in excess of, genetic predisposition.

As diagnosis and treatment of CRC have improved, the study of the pathogenesis of colorectal neoplasia has increased. The most frequent precursor of CRC is the adenoma. As a proportion of adenomas, those of large size, with villous architecture and high grade dysplasia often progress to invasive adenocarcinoma, and this progression is associated with accumulation of mutations and other genetic and epigenetic changes. In the effort to understand the mechanisms and causes of colorectal cancer development, molecular genetic analyses have identified a variety of molecular changes and protein targets involved in colorectal tumourigenesis. The greater understanding of genetic, epigenetic and expression changes that occur during the development and progression of CRC has shown that these neoplasms do not comprise a single disease. Instead, colorectal cancers comprise a collection of distinct and independent neoplastic pathways, such as those pathways displaying chromosomal instability (CIN), microsatellite instability (MSI) or gene promoter activity changes due to the epigenetic phenomenon of methylation at CG dinucleotides (referred to as CIMP: CpG island methylation phenotype, whereby CpG describes dinoculeotides of cytosine and guanosine, separated by the characteristic phosphate group in the DNA structure). Each pathway subtype is characterised by individual genetic and molecular characteristics (Poulogiannis,

Ichimura, Hamoudi, Luo, Leung, Yuen, Harrison, Wyllie & Arends, 2010; Poulogiannis, McIntyre, Dimitriadi, Apps, Wilson, Ichimura, Luo, Cantley, Wyllie, Adams & Arends, 2010). Dietary constituents have been studied in relation to the major genetic and molecular changes occurring in CRC development, including alterations in the proto-oncogenes, *K-RAS* and *BRAF* and the tumour suppressor genes *p53* and *APC*. Many studies have analysed a wide variety of dietary components in an effort to elucidate which, if any, dietary constituents may contribute to their mutation in CRC progression. Furthermore, in addition to these genetic lesions, the epigenetic phenomenon of CIMP and MSI have similarly been analysed in relation to dietary constituents.

This review is intended to summarise the currently available literature describing the associations between the molecular genetic changes seen most prevalently in colorectal cancer and dietary intakes. This report does not attempt to assess dietary associations with total CRC incidence. The objective is to highlight consensus observations, where several sources of data exist, suggestive of causative or protective effects of dietary constituents regarding specific molecular genetic changes frequently observed in colorectal neoplasia. Throughout, an emphasis is placed on the number of cases analysed in individual studies, but notably absent are descriptions of odds ratios, hazard ratios or p-values. Throughout, all associations discussed are statistically significant (all $p \leqslant 0.05$). However, due to the varying methodology of data collection and statistical analysis across studies, the inclusion of differing variables in adjusted models and the lack of consensus regarding the degree to which analyses should be adjusted following multiple statistical tests, detailed statistical aspects are not discussed. In order for an assessment to be made of the potential statistical power of each analysis, the number of cases involved in each study is instead highlighted when a statistically significant association is discussed. Full details of all statistical analyses can be found in the original reports, referenced in the text and listed at the end of the chapter.

2. Dietary influences on the major genetic and epigenetic perturbations leading to colorectal cancer development and progression

2.1 *K-RAS* and *BRAF* in colorectal cancer: the MAPK signalling pathway

Mitogen activated protein kinase (MAPK) signal transduction pathways are present in all eukaryotes, six versions of which have been distinguished in mammals (Robinson & Cobb, 1997). MAPK signal propagation is responsible for regulating a variety of cellular processes, which include potentially pro-tumourignenic properties such as proliferation, apoptosis and transformation (Arends et al., 1993; 1994; Peyssonnaux & Eychene, 2001; Robinson & Cobb, 1997). The best characterised of these pathways is the LIGAND RECEPTOR-RAS-RAF-MEK-ERK pathway (Figure 1), which consists of core modules including the RAS and RAF proteins. Although three *RAS* genes have been identified (*H-RAS*, *N-RAS* and *K-RAS*), the *K-RAS* gene is the only one mutated at significant frequency in CRC (Bos, 1989). Similarly, of the three *RAF* genes identified (*ARAF*, *BRAF* and *CRAF/RAF-1*), only the *BRAF* gene is mutated at significant frequencies in human cancers (Fransen et al., 2004).

Experimental mouse models have provided direct evidence that mutated *K-RAS* genes expressed in the intestinal epithelium do not significantly initiate intestinal adenoma growth, but they can cooperate either with other mutant genes or carcinogens to accelerate intestinal tumour formation (Luo et al., 2007; 2009; Luo, Poulogiannis, Ye, Hamoudi & Arends, 2011;

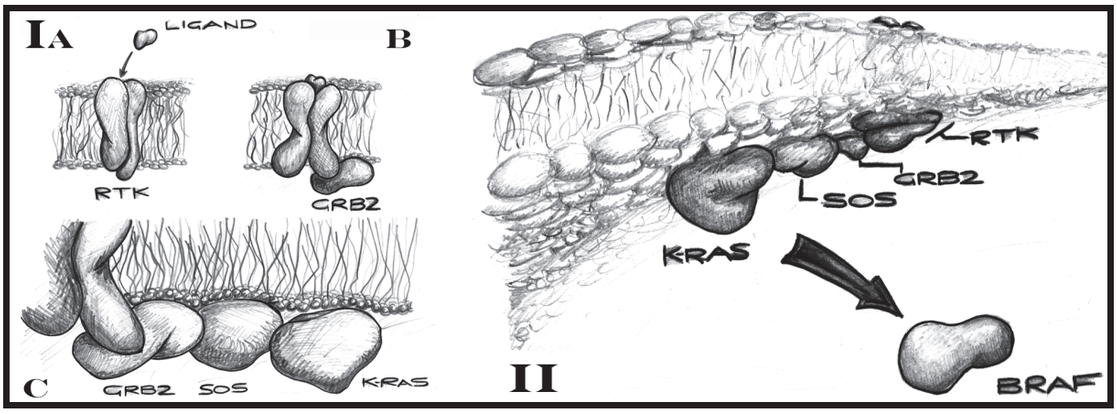


Fig. 1. **RAS, RAF and the MAPK signalling pathway: frequently perturbed in colorectal neoplasms.** Initially, RAS is inactive in a RAS-GTP bound state. **I:** Initiation of signalling through the MAPK pathway occurs at the plasma membrane. Upon extracellular ligand binding to membrane receptor tyrosine kinases (RTK), such as epidermal growth factor binding to the epidermal growth factor receptor (IA), receptor conformational change gives rise to receptor autophosphorylation. Subsequently, src-homology 2 (SH2) domains present in the GRB2 adaptor protein bind the phosphate moieties on the activated receptor (IB). Src-homology 3 (SH3) domains in GRB2 bind proline-rich motifs present in son of sevenless (SOS), localising SOS to the inner surface of the plasma membrane. SOS, a guanine nucleotide exchange factor (GEF) interacts with RAS proteins, catalysing the exchange of GDP for GTP, thus activating RAS to a RAS-GTP state (IC). **II:** Upon activation, RAS phosphorylates cytosolic RAF. The resulting activation of RAF in turn phosphorylates cytosolic MEK, which then phosphorylates ERK, leading to induction and repression of distinct transcription programmes, promoting cell proliferation and modulating cell death by apoptosis, among other processes. The vast majority of mutations in the *K-RAS* or *BRAF* genes are in distinct hotspot regions: *K-RAS* at codons 12 and 13, and also, but much more infrequently at codons 61 and 146 (Forbes et al., 2008). Additionally, mutations observed at lower prevalences at other sites in the gene have been described and their functional significance determined (Naguib, Wilson, Adams & Arends, 2011). Mutations in *BRAF* occur most frequently at codons 463-468 and codon 600 (Forbes et al., 2008). Activating mutations in the *K-RAS* and *BRAF* genes render their protein products constitutively active, leading to increased transduction through this signalling axis. Additionally, mutationally active *K-RAS* can also propogate signalling through other pathways, including the PI3K/AKT axis.

Luo, Poulogiannis, Ye, Hamoudi, Zhang, Dong & Arends, 2011). *K-RAS* mutations are observed 20-50% of sporadic human CRC and *BRAF* mutations are observed in 5-15% of CRC (Forbes et al., 2008). The high frequencies at which *K-RAS* and *BRAF* mutations are observed in CRC has prompted several analyses of dietary intakes in relation to these genetic lesions.

2.1.1 *K-RAS* mutation and meat consumption

Specific types of meat consumption have been identified as associated with general CRC incidence (Norat et al., 2005; Santarelli et al., 2008) with plausible mechanisms postulated as to the manner in which these consumptions may influence colorectal carcinogenesis (Kuhnle & Bingham, 2007; Kuhnle et al., 2007). Consequently, several studies have attempted to identify

the nature of these associations in relation to *K-RAS* mutations. Some reports have identified associations with meat consumption and *K-RAS* mutation, although not all.

A single study analysing 390 *K-RAS* wildtype and 218 *K-RAS* mutated CRC identified an increased consumption of beef with higher incidence of *K-RAS* wildtype colonic cancers (Brink, Weijenberg, de Goeij, Roemen, Lentjes, de Bruïne, Goldbohm & van den Brandt, 2005). In this same report, a reduction in pork consumption was found to be linked to reduced frequency of both colonic and rectal cancers harbouring mutated *K-RAS*. Another report, assessing *K-RAS* mutations and diet in 155 *K-RAS* wildtype and 41 *K-RAS* mutated CRC, identified an increased white meat consumption associated with higher incidence of *K-RAS* mutated CRC (Naguib et al., 2010). Although positive associations were identified in these two analyses, there appears to be little consistency between these independent findings. The report by Naguib and colleagues also analysed red and processed meat consumption in relation to mutation status and found no statistically significant association between the two, although, beef consumption was not tested independently of other meat types, as in the report by Brink and co-workers. The study by Naguib and colleagues did not test pork consumption in isolation: this meat type was included in the 'red' or 'processed' meat categories. Similarly, Brink and coworkers did not identify an association between white meat and increased incidence of *K-RAS* mutations. This analysis tested the consumption of chicken in isolation, not in a combined 'white meat' category containing other meat types, such as turkey etc.

Notwithstanding the identified statistically significant associations between meat consumption and *K-RAS* mutation status described above, the majority of studies which have attempted to address this question have failed to identify any link between meat intake and the mutation status of this gene. An analysis testing 67 *K-RAS* wildtype and 39 *K-RAS* mutated CRC assessed animal protein intake and found no link between this and *K-RAS* mutation status (Bautista et al., 1997) although clearly, 'animal protein' as a variable makes no distinction between meat types and is an assessment of protein, not animal product, consumption. A large analysis testing 971 *K-RAS* wildtype and 457 *K-RAS* mutated CRC (Slattery et al., 2000) identified no assocaition between total *K-RAS* mutations and meat intake. A small study (28 wildtype, 15 mutated rectal cancers) failed to identify an association between red meat intake and *K-RAS* mutation (O'Brien et al., 2000). An assessment of a larger cohort of rectal cancers (535 *K-RAS* wildtype and 215 *K-RAS* mutated) corroborated this observation of lack of association with red meat intake and rectal cancer (Slattery, Curtin, Wolff, Herrick, Caan & Samowitz, 2010).

In addition to colorectal cancers, pre-cancerous adenomas have also been tested in order to identify dietary assocaitions with *K-RAS* mutation status in the early stages of colorectal neoplasia. An assessment of 558 *K-RAS* wildtype and 120 *K-RAS* mutated adenomas failed to identify an association between red meat intake and mutation status (Martínez et al., 1999). Another study, testing 453 *K-RAS* wildtype and 81 *K-RAS* mutated adenomas also failed to identify a statistically significant association between red meat, processed meat or poultry and *K-RAS* mutation status (Wark et al., 2006).

Published reports assessing *K-RAS* mutation status in CRC in relation to meat intakes provide limited evidence to suggest that total *K-RAS* mutations are either positively or negatively associated with meat consumption. The majority of studies have categorised meat types according to shared properties (such as haem content or preservation methods) and have generally failed to identify links between these groups and *K-RAS* mutation status. It is

Study	*K-RAS* WT CRC/RC/adenomas	*K-RAS* mutated CRC/RC/adenomas	dietary association
Bautista *et al* 1997	CRC: 67	CRC: 39	↑ MUFA with *K-RAS* mutation, ↓ calcium with *K-RAS* mutation
Bongaerts *et al* 2006	CRC: 385	CRC: 193	no association between alcohol and *K-RAS* mutated or wildtype cancers
Brink *et al* 2004	CRC: 390	CRC: 218	↑ PUFA (specifically linoleic acid) with *K-RAS* mutated colonic, but not rectal, cancers
Brink *et al* 2005	CRC: 390	CRC: 218	↑ folate reduced risk of *K-RAS* mutated rectal, not colonic, cancer in men only
Brink *et al* 2005	CRC: 390	CRC: 218	↑ beef, ↓ pork with *K-RAS* wildtype colonic tumours, ↓ pork with *K-RAS* wildtype rectal tumours
Laso *et al* 2004	CRC: 68	CRC: 49	*K-RAS* codon 12 mutation was associated with ↓ vitamin A, B1, D and iron
Martinez *et al* 1999	Adenomas: 558	Adenomas: 120	↑ folate reduced risk of developing *K-RAS* mutated adenomas
Naguib *et al* 2010	CRC: 155	CRC: 41	↑ white meat consumption with *K-RAS* mutation
O'Brien *et al* 2000	RC: 28	RC: 15	no association between red meat consumption and *K-RAS* mutation
Schernhammer *et al* 2008	CRC: 427	CRC: 242	no association between folate intake and prevalence of *K-RAS* mutated or wildtype cancers
Slattery *et al* 2000	CRC: 971	CRC: 457	↓ cruciferous vegetables with reduced risk of *K-RAS* mutation
Slattery *et al* 2010	RC: 535	RC: 215	no association between calcium and vitamin D and *K-RAS* mutation
Slattery *et al* 2010	RC: 535	RC: 215	↑ vegetables and dietary fibre with a reduced risk of *K-RAS* mutations
Wark *et al* 2006	Adenomas: 453	Adenomas: 81	↓ MUFA and ↑ vitamin B2 associated with *K-RAS* mutation

Table 1. Summarised description of literature analysing *K-RAS* mutations in colorectal neoplasms (case numbers provided) in relation to dietary intakes and the statistically significant findings described. *WT*: wildtype, *CRC*: colorectal cancer, *RC*: rectal cancer, *MUFA*: monunsaturated fatty acid, *PUFA*: polyunsaturated fatty acid, ↑ and ↓ denote an increase or decrease in consumption respectively.

plausible that if specific meat types, as suggested in at least one study (Brink, Weijenberg, de Goeij, Roemen, Lentjes, de Bruïne, Goldbohm & van den Brandt, 2005), are linked to the incidence of *K-RAS* mutated CRC, that grouping of meat types together may have failed to identify associations where they existed. However, in practical terms, it should be noted that similarities in the composition of meat types, such as in terms of haem content, a postulated carcinogen intermediate (Kuhnle & Bingham, 2007), justify a grouping of types in order to minimise multiple statistical testing and to test consumption levels large enough to be likely to affect bowel carcinogenesis.

Several reports have analysed the relationship between base changes at specific positions in the *K-RAS* gene, types of mutations (i.e. transition *versus* transversion) or specific types of base changes (i.e. G→A) in relation to meat intakes. It is entirely plausible that the nature of the mutation, not the gene in which it arises, is linked to dietary constituents. However, due to the very limited number of studies instigated with objectives of such an analysis, and the often low numbers of different mutation subgroups existent in the studies which do attempt such an assessment rendering lower statistical power, such analyses are not discussed in this review.

2.1.2 *K-RAS* mutation and folate consumption

Several studies have described an association between folate intake and the prevalence of *K-RAS* mutations in CRC. A report analysing 390 *K-RAS* wildtype and 218 *K-RAS* mutated CRC identified an increased consumption of folate associated with a reduced prevalence of *K-RAS* mutated rectal, but not colonic, cancers in males only (Brink, Weijenberg, de Goeij, Roemen, Lentjes, de Bruïne, van Engeland, Goldbohm & van den Brandt, 2005). Testing in this study demonstrated that in the male participants of this cohort, increased intake of folate was linked to reduced prevalence of rectal cancer incidence, however, this link, when considering mutation status, seemed only to reduce the risk of *K-RAS* mutated rectal cancers. A large analysis of colorectal adenomas (558 wildtype, 120 *K-RAS* mutated) also identified increased folate intake associated with a reduced incidence of *K-RAS* mutation (Martínez et al., 1999).

However, in addition to these positive associations in relatively large cohorts, several other studies have failed to identify a link between folate intake and *K-RAS* mutation status in

colorectal neoplasms. Reports describing the testing of 67 *K-RAS* wildtype and 39 *K-RAS* mutated CRC (Bautista et al., 1997), 68 *K-RAS* wildtype, 49 *K-RAS* mutated CRC (Laso et al., 2004), 155 *K-RAS* wildtype, 41 *K-RAS* mutated CRC (Naguib et al., 2010), 427 *K-RAS* wildtype, 242 *K-RAS* mutated CRC (Schernhammer, Giovannuccci, Fuchs & Ogino, 2008), 971 *K-RAS* wildtype 457 *K-RAS* mutated CRC (Slattery et al., 2000) and 453 *K-RAS* wildtype, 81 *K-RAS* mutated adenomas (Wark et al., 2006) failed to identify folate intake as associated with *K-RAS* mutation status.

Increased consumption of folate offering some degree of protection against *K-RAS* mutation was observed in two independent studies. The failure to confirm this link in many other reports may potentially be explained several ways. Firstly, many of the studies described which identified no link between *K-RAS* mutation and folate intake contained relatively few mutated samples (<100). It is plausible that in these instances too few cases were analysed to detect any association, although this does not explain the studies which failed to identify a link using relatively large sample sets (Schernhammer, Giovannuccci, Fuchs & Ogino, 2008; Slattery et al., 2000). Secondly, some dietary constituents have been described to affect folate utilisation, such as alcohol (Eichholzer et al., 2001; Freudenheim et al., 1991). It may be possible that the protective effect of folate against *K-RAS* mutation is only prevalent in the context of certain dietary patterns, possibly explaining why associations are not observed in all epidemiological studies. Finally, Martinez and colleagues identified an increased protective effect against *K-RAS* mutation as provided by supplement derived intake relative to natural dietary intake of this macronutrient (Martínez et al., 1999). The nature of folate consumption, i.e. bioavailablilty, may also determine the degree to which it offers a protective effect in colorectal carcinogenesis.

Although not observed in every analysis, increased intake of folate is associated with a reduced prevalence of total CRC incidence, which is observed in approximately half of the analyses testing this link (Eichholzer et al., 2001). It is probable, that at least to a limited degree and in certain circumstances, that this may be due to the ability of folate to protect against *K-RAS* mutation during development of colorectal neoplasia.

2.1.3 *K-RAS* mutation and fat consumption

Consumption of several forms of fat intake have been described to affect the prevalence of *K-RAS* mutations in CRC. However, there is no consensus in the literature to date, regarding both the manner of the association and type of fat involved. Independent studies have identified monounsaturated fatty acid (MUFA) consumption as associated with the prevalence of *K-RAS* mutations in CRC. One report, analysing 67 *K-RAS* wildtype and 39 *K-RAS* mutated CRC, identified an increased MUFA consumption as linked to an increased prevalence of *K-RAS* mutated CRC (Bautista et al., 1997). MUFA, mostly derived from olive oil in this population, reduced the risk of CRC harbouring wildtype *K-RAS*, but offered no protection against *K-RAS* mutated cancers. However, contradictory findings of an increased MUFA consumption being associated with a higher prevalence of *K-RAS* wildtype neoplasia in a study assessing adenomas (453 wildtype, 81 mutated) (Wark et al., 2006) challenges the observation made by Bautista and co-workers. Other published reports have failed to identify any link between *K-RAS* mutation status and MUFA intake (Brink et al., 2004; Laso et al., 2004; Naguib et al., 2010; Slattery et al., 2000; Slattery, Curtin, Wolff, Herrick, Caan & Samowitz, 2010).

In addition to these observations, one report describes an increase in polyunsaturated fatty acids (PUFA), specifically linoleic acid, as associated with increased prevalence of *K-RAS* mutated colonic, but not rectal, cancers (Brink et al., 2004). However, this association with PUFA has not been identifed in any other report (Bautista et al., 1997; Laso et al., 2004; Naguib et al., 2010; Slattery et al., 2000; Slattery, Curtin, Wolff, Herrick, Caan & Samowitz, 2010; Wark et al., 2006).

Taken together, the published data describing the association of dietary fats with *K-RAS* mutations have failed to identify a convincing association, and have generated conflicting results. Presently, the evidence suggesting that the *K-RAS* mutation status of colorectal neoplasia may be affected by fat intakes is weak: the limited data available suggest that the mutation status of this gene is largely independent of this dietary consumption. It should be noted however, that although fat intake itself is probably not associated with this mutation type, increased body mass index (BMI), which may be associated with fat intake, is associated with overall CRC risk.

2.1.4 *K-RAS* mutation and other dietary constituents

The mutation status of *K-RAS* in CRC has also been linked to several other dietary variables in addition to meat, folate and fat. Testing of 971 *K-RAS* wildtype and 457 *K-RAS* mutated CRC identified an increased risk of *K-RAS* mutations with reduced consumption of cruciferous vegetables (Slattery et al., 2000). Another analysis of rectal cancers (535 wildtype, 215 mutated) identified a reduced incidence of *K-RAS* mutated rectal cancers with increased vegetable and fibre intake (Slattery, Curtin, Wolff, Herrick, Caan & Samowitz, 2010). Although corroborative, these two analyses were performed on the same test cohort and are yet to be identified in other independent populations. In this cohort at least, the data of Slattery and colleagues suggest that increased vegetable intake reduced the prevalence of *K-RAS* mutations in CRC, with an overt association identified in rectally located neoplasia.

Increased vitamin B2 intake has been identified to reduce the prevalence of adenomas harbouring *K-RAS* mutations. In an analysis of 453 *K-RAS* mutated and 81 *K-RAS* wildtype pre-cancerous adenomas an inverse association suggested a protective effect against *K-RAS* mutated adenomas. This protection was not found in relation to the prevalence of *K-RAS* wildtype adenomas (Wark et al., 2006). This association has not been identified in cohorts testing colorectal cancers.

Some dietary intakes have been repeatedly tested with no link to the prevalence of *K-RAS* mutation in CRC having been identified, notably alcohol. Many studies have included assessment of this dietary factor, with some studies analysing alcohol consumption independent of any other dietary factors (Bongaerts et al., 2006).

In summary, current literature describing the assessment of *K-RAS* mutation status in colorectal neoplasia has identified many associations with dietary intakes (summarised in Table 1). Very few of these associations have been repeatedly identified in independent cohorts, making assessment of their general validity challenging. Presently, few dietary components seem to be strongly linked to *K-RAS* mutation status in CRC across many populations, environments and genetic backgrounds. Furthermore, it is problematic to directly compare different studies. Other than folate, which has been described by the World Cancer Research Fund as having a 'limited' protective effect against CRC, which may impart this limited protection through reduced prevalence of *K-RAS* mutation, there is a lack of strong

evidence to firmly suggest any other dietary intakes affect the prevalence of *K-RAS* mutations in CRC.

2.1.5 *BRAF* mutations and dietary associations

Relative to *K-RAS*, far fewer data exist describing the association between *BRAF* mutations in CRC and dietary influences. A prospective study involving 186 colorectal cancers, of which 29 harboured *BRAF* mutations, analysing meat, fruit and vegetable, fat, vitamin and macronutrient intakes identified no potential dietary associations with *BRAF* mutation in CRC (Naguib et al., 2010).

Other analyses have centred on analysing dietary constituents which may act as methyl group donors, such as folate, or vitamins, such as B6 and B2, which function as co-factors in the pathway responsible for DNA methylation (de Vogel et al., 2008; Kim, 2005). Based on observations that *BRAF* mutation has been linked previously to the CIMP phenotype (Lee et al., 2008; Samowitz et al., 2005; Velho et al., 2008) and has been linked to 60-80% of CRC demonstrating the highest levels of CIMP with concurrent MSI (Kambara et al., 2004; Samowitz et al., 2005), this mutation type may be influenced by dietary factors thought to influence DNA methylation. One such analysis used data and tissue samples from 648 individuals, of which 101 harboured CRC with mutations in the *BRAF* gene. This report identified a positive association between *BRAF* mutation in males and the highest tertile of folate consumption (de Vogel et al., 2008). This same report also identified an inverse correlation between methionine intake, as well as no association between vitamin B2 and alcohol consumption and *BRAF* mutations in the male portion of the cohort. In the female cohort members, no dietary consumptions were identified which were associated with *BRAF* mutations. An additional assessment of 86 *BRAF* mutated and 300 *BRAF* wildtype colonic cancers failed to identify an association between alcohol, folate, vitamins B6 and B12 or methionine consumption and *BRAF* mutation status (Schernhammer et al., 2011).

Another study population, of which 1108 cases of CRC were assessed for the presence of *BRAF* mutations, identified no associations between the 114 cancers harbouring this genetic lesion and intake of either vitamins B6, B12, folate, methionine or fibre consumptions, when compared with non-cancerous controls (Slattery et al., 2007). Similarly, the determination of *BRAF* mutation status in 189 CRC cases in another study cohort identified no associations between mutations in this gene and plasma levels of folate, vitamin B12 and homocysteine (Van Guelpen et al., 2010).

At present, few analyses of dietary intake in relation to the incidence of *BRAF* mutations in CRC have been attempted, and the majority of the limited data which do exist generally fail to identify strong associations between CRC harbouring *BRAF* mutations and any dietary constituent. In only one study to date, limited, sex specific dietary associations with *BRAF* mutation have been identified (de Vogel et al., 2008), but these observations are yet to be validated and corroborated in other studies.

The lack of identification of any of dietary component associated with *BRAF* mutation in CRC may have several causes. Primarily, only one study, analysing a very limited number of *BRAF* mutated tumours (n=29) has attempted a broad analysis of many dietary factors (Naguib et al., 2010). The remaining limited data has involved anlaysis of only a selected spectrum of dietary components hypothesised to be involved in the DNA methylation process. The limited scope of these studies in terms of dietary factors tested does not exclude the possibility that other

dietary factors may be associated with *BRAF* mutated CRC. Secondly, *BRAF* is identified at higher frequency in CRC demonstrating CIMP and MSI. Definitive evidence is yet to be provided describing the order in which tumours displaying CIMP and MSI acquire these instabilities and when *BRAF* mutations are acquired during progression. It is plausible that mutation in the *BRAF* gene is secondary to the acquisition of these global genomic alterations. As such, the question of diet and any relationships with this mutation may be redundant, if following the acquisition of CIMP and MSI status, *BRAF* mutation may arise independent of dietary influences. Thirdly, the limited number of studies available addressing the question of dietary associations and *BRAF* mutation may be too few in number to identify any dietary associations with this lesion, or, the majority of the studies performed are correct and that in this instance, dietary components do not affect the prevalence of *BRAF* mutations in CRC.

2.2 *p53* mutations in colorectal cancer

The *p53* tumour suppressor gene is the most commonly mutated gene in all human cancers, mutated in approximately 50% of human malignancies, including 50-60% of CRC (Forbes et al., 2008). Subsequent to its activation following DNA damage, oxidative stress or other cellular insults, wildtype p53 protein accumulates in the cell nucleus and acts as a transcription factor, capable of activating and suppressing transcription programmes leading to cell cycle arrest, DNA damage repair and apoptosis (Aylon & Oren, 2011; Bourdon et al., 2003). As such, perturbation of the normal role of p53 is highly selected for in cancer cells. The high prevalence of *p53* mutation in CRC, notably in later stage cancers, has led to various studies of mutations of this gene in the context of dietary consumptions.

2.2.1 *p53* mutations and dietary associations

Mutations in the *p53* gene have been linked to a variety of dietary intakes. Low folate and vitamin B6 intakes have been linked to p53 over-expressing cancers (Schernhammer, Ogino & Fuchs, 2008). This report, analysing 143 p53 over-expressing and 256 colonic cancers demonstrating low or absent p53 expression used an immunohistochemical (IHC) analysis to assess p53 accumulation following mutation. p53 over-expression or accumulation is the result of reduced protein degradation, mostly due to point mutations in the *p53* gene, greatly increasing the half-life of the gene's protein product (Melhem et al., 1995). This fast method of assessment of a range of activating *p53* mutations should be interpreted with some caution however, as less commonly observed mutations giving rise to truncated protein, such as those introducing premature *stop* codons, are not identified using this method. The observation linking low folate intake to an increased prevalence of cancers of the colon exhibiting over-expression of p53 is yet to be corroborated. Two reports using DNA sequencing, testing 62 *p53* mutated and 123 *p53* wildtype CRC (Park et al., 2010) and 686 *p53* mutated and 772 *p53* wildtype colonic cancers (Slattery et al., 2002), identified no link between p53 status and folate intakes. Little or no apparent other data exist describing vitamin B6 intakes and possible relationships with *p53* mutation status.

Specific meat intakes have been linked to *p53* mutation status in several independent studies. One report by Park and colleagues identified an increased consumption of red and total meat (all types, including poultry) as associated with increased prevalence of *p53* mutations in CRC, however, this was only present in advanced stage CRC (Dukes' C or D), not in those of less advanced stages (Dukes' A or B) (Park et al., 2010). In addition to this, an assessment by

Slattery and co-workers identified high glycaemic load, increased red meat, increased fast food and increased trans fatty acid intakes as associated with increased prevalence of *p53* mutations in colonic cancers (Slattery et al., 2002). These two independent studies suggest that red meat in particular may promote mutations in *p53* in neoplasia of the large intestine. However, these data do not completely overlap: the study by Park and colleagues only found this association in advanced stage cancers and the report by Slattery and co-workers assessed only colonic, not rectal cancers. Opposed to the above observations of meat intakes promoting *p53* mutations in CRC, an IHC based analysis (73 p53 over-expressing, 90 p53 absent CRC) identified increased beef consumption as associated with reduced prevalence of p53 over-expressing cancers (Freedman et al., 1996). Further data are needed to evaluate the potential association of meat, and meat types, with *p53* mutations in CRC, with particular emphasis on cancer location and stage.

In a study of colonic cancers assessing both p53 expression and *p53* gene mutations, total and saturated fats were identified as linked to tumours not over-expressing p53 or harbouring gene mutations (Voskuil et al., 1999). Of the 185 colonic cancers tested in this study, 81 displayed p53 overexpression by IHC, of which 59 were found to harbour mutations in the sequenced region (exons 5-8) of these cancers. Mutations in *p53* were not found to be linked to total fat intake in other reports assessing either CRC as a general subgroup (Park et al., 2010) or rectal cancers in particular (Slattery, Curtin, Wolff, Herrick, Caan & Samowitz, 2010). An analysis of 340 *p53* mutated and 410 *p53* wildtype rectal cancers reported an increased consumption of vegetables, whole grains and fibre associated with reduced prevalence of *p53* mutation (Slattery, Curtin, Wolff, Herrick, Caan & Samowitz, 2010). Conversely, a high intake of refined grains was found to increase the prevalence of rectal cancer harbouring *p53* mutations. Increased intakes of cruciferous vegetables have also been described to be associated with reduced prevalence of p53 over-expressing CRC (73 p53 over-expressing CRC, 90 p53 absent CRC) (Freedman et al., 1996). The observation of increased vegetable intakes associated with reduced frequency of *p53* mutations in CRC was not observed in another study analysing general CRC (Park et al., 2010). Fibre was not observed to be associated with a protective effect in analyses combining colonic and rectal cancers (Park et al., 2010) or assessing colonic cancers in isolation (Slattery et al., 2002; Voskuil et al., 1999).

Alcohol intakes and *p53* mutation status in CRC have been assessed in several reports. A study analysing 340 *p53* mutated and 410 *p53* wildtype rectal cancers identified increased beer consumption as being associated with higher prevalence of *p53* mutations when compared with non-beer drinkers (Slattery, Wolff, Herrick, Curtin, Caan & Samowitz, 2010). No associations between alcohol intakes and *p53* mutation status have been identified in several analyses of colonic cancers (Schernhammer, Ogino & Fuchs, 2008; Voskuil et al., 1999), however, neither of these studies assessed specific alcoholic beverages, just total alcohol intake. Total alcohol intake was found to be linked to increased prevalence of *p53* mutations in CRC of advanced Dukes' stage (C and D), but not in CRC of less advanced stage (Dukes' A or B) (Park et al., 2010). Another report analysing Dukes' stage C cancers by IHC (42 p53 over-expressing CRC, 65 p53 absent CRC) did not identify total alcohol intake as linked to p53 expression status (Zhang et al., 1995).

Presently, the limited data on *p53* mutation status in CRC and dietary intakes are inconsistent. As a result, several consumptions have been linked to *p53* mutation status but none have been corroborated by other studies performing a similar assessment in an independent cohort.

Further evidence is needed to substantiate these isolated observations. Future studies should focus on the analysis of the potential association of vegetable and meat intakes in relation to p53 status as several data exist suggesting a possible link between these intakes and p53 aberrations, although contrary observations have been published.

2.3 *APC* mutations in colorectal cancer

The *adenomatous polyposis coli* (*APC*) gene is one of the most frequently mutated genes in colorectal cancer (Sjöblom et al., 2006; Wood et al., 2007), with some studies reporting 50-80% of CRC harbouring mutations in this gene (Forbes et al., 2008). The majority of mutations identified in CRC in the *APC* gene are located in exon 15 in the central third of the coding sequence, the *mutation cluster region*, which corresponds to the β-catenin-binding region of the protein (Goss & Groden, 2000). Mutations in *APC* most frequently result in truncation of the protein, corresponding with a reduction in the ability of APC to bind β-catenin (Figure 2). In addition to its role as a modulator of WNT pathway signalling, APC also has a role in mitosis and cytokinesis: cells harbouring truncated APC undergo abnormal chromosomal segregation and may develop aneuploidy (Ceol et al., 2007). Wildtype APC functions as a regulator of apoptosis, differentiation and migration and functions during cell division (Ceol et al., 2007; Fodde et al., 2001; Goss & Groden, 2000).

Although mutations in other genes, such as *p53*, may be almost as frequent as those in *APC* in CRC, *APC* mutations seem to be particularly prevalent from the earliest stages of CRC initiation and progression. Dysplastic aberrant crypt foci (ACF), monocryptal or oligocryptal adenomas, which are the lesions considered to be the earliest forms of colorectal neoplasia, frequently display *APC* mutations (Jen et al., 1994) and can develop into CRC through the adenoma-carcinoma sequence (Suehiro & Hinoda, 2008; Takayama et al., 1998). Intriguingly, the more frequently occurring heteroplastic ACF, which possess limited, if any, potential to develop to malignancy, very rarely harbour *APC* mutations but frequently exhibit *K-RAS* mutations (Jen et al., 1994). These data suggest that initiating genetic lesions in CRC determine malignant potential, and that if the initial mutations occur in the *APC* gene, there is a high probability of subsequent adenoma formation. In concordance with observations in dysplastic ACF, *APC* mutations are very frequently observed in colorectal adenomas (Kinzler & Vogelstein, 1996) and when inherited as germline *APC* mutations allow formation of hundreds of colorectal adenomas in the Familial Adenomatous Polyposis Coli syndrome. Hence, there have been several analyses of APC mutations in CRC relation to dietary intakes, with the purpose of identifying links between this early genetic lesion and dietary carcinogens.

2.3.1 *APC* mutations and dietary associations

APC mutations have been linked to several dietary constituents. One report, analysing 121 *APC* wildtype and 63 *APC* mutated colonic cancers, identified alcohol as inversely associated with *APC* mutated and positively associated with *APC* wildtype cancers (Diergaarde, van Geloof, van Muijen, Kok & Kampman, 2003). Additionally, red meat, fish and fat, notably unsaturated fat, were shown to be associated with development of *APC* mutated colonic cancers. Conversely, another report assessing 347 *APC* wildtype CRC and 184 *APC* mutated CRC identified increased consumption of saturated fat, but not unsaturated fats, as associated with *APC* mutated rectal cancers (Weijenberg et al., 2007). Furthermore, the analysis by

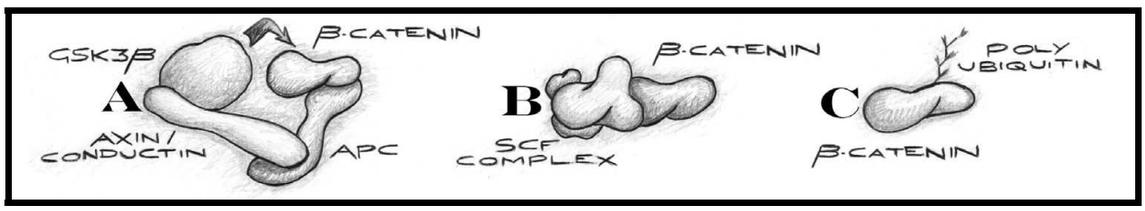


Fig. 2. **APC and the WNT signalling pathway**. **A:** In the absence of WNT signal, free β-catenin is bound by APC, in a complex with axin/conductin and glycogen synthase kinase 3β (GSK3β) and this complex acts as a scaffold, bringing β-catenin into close proximity with GSK3β. This results in GSK3β mediated phosphorylation of β catenin. **B:** Phosphorylated β-catenin is recognised by the SCF complex and is polyubiquitinated. **C:** Polyubiquitinated β-catenin is recognised by the proteasome and degraded. In the absence of WNT signalling, β-catenin is largely degraded, thus preventing β-catenin nuclear accumulation and subsequent co-activation of transcription programs. Upon binding of WNT ligand to membrane-located receptors, a subsequent signalling cascade prevents formation of the APC-axin-conductin-GSK3β complex. As a result, β-catenin avoids degradation and can translocate to the nucleus where it co-activates transcription of target genes, such as *c-myc*.

Weijenberg and co-workers identified specific types of *APC* wildtype CRC (i.e. those harbouring *K-RAS* mutations and showing no loss of MLH1 expression [see 2.4.2]) as being linked to increased intake of linoleic acid, a polyunsaturated fatty acid.

A further study has identified increased consumption of folate associated with reduced prevalence of *APC* wildtype colonic cancer, but increased prevalence of *APC* mutated colonic cancers in males (de Vogel et al., 2006). These associations were not observed in rectal cancers of men or in either colonic or rectal female cancer cases. This analysis, studying 347 *APC* wildtype CRC and 182 *APC* mutated CRC, also identified increased vitamin B2 and iron intakes in men associated with colonic cancers harbouring *APC* mutations compared with those men with colonic cancer not harbouring *APC* mutations.

These analyses are difficult to compare, notably as Diergaarde and colleagues did not stratify cases by sex or cancer location, which may possibly explain the lack of association between folate intake and *APC* mutation status in their report. The study by Diergaarde and co-workers did not analyse iron or vitamin B2, and de Vogel and colleagues did not assess meat and fish intakes. Alcohol association with *APC* mutation status was not observed in the testing by de Vogel and co-workers. Assessed in conjunction, these studies do not corroborate each other as direct comparisons are difficult to make.

Further analysis of *APC* mutation status has been performed in the context of specific meat intakes. In a study of 347 *APC* wildtype CRC and 184 *APC* mutated CRC, increased processed meat consumption was linked to an increased prevalence of *APC* mutated colonic cancers (Lüchtenborg et al., 2005). Additionally, increased beef consumption was linked to increased frequency of *APC* wildtype colonic cancers. Rectal cancers without *APC* mutations were found to be more prevalent amongst those with increased consumption of other meat types, which included horsemeats, lamb and mutton among other products. This detailed analysis of *APC* mutation status in the context of very specific meat types, with both positive and negative associations having been identified, is yet to be corroborated by similarly detailed meat-type subgroups testing in additional studies. This report does suggest however, that meat classification is important when testing for associations with *APC* mutations. In this

context, these observations partially confirm the increased consumption of general red meat that was observed to be associated with an increased risk of *APC* mutated CRC in the report by Diergaarde and co-workers.

In addition to reports assessing *APC* mutation status relative to dietary intakes in CRC, a single study has assessed these relationships in colorectal adenomas (Diergaarde et al., 2005). This analysis of 117 *APC* wildtype adenomas and 161 *APC* mutated colorectal adenomas identified a high intake of red meat and fat as associated with increased prevalence of *APC* wildtype adenomas. These observations are intriguing as identification of increased consumptions of certain red meat types being specifically associated with certain *APC* wildtype CRC has been described previously (Lüchtenborg et al., 2005).

Taken together, the available data describing *APC* mutations in CRC in relation to dietary intakes are too few and inconsistent to draw any strong conclusions. However, several analyses have identified certain meat consumptions as linked to either colonic or rectal cancers with a particular *APC* mutation status. These observations, although not in full agreement, indicate that certain red meat types, determined by both animal origin and preparation method, may affect the prevalence of mutation in *APC* in CRC. Further assessment of these particular dietary associations are warranted to determine the relationship between *APC* mutation status and specific red meat consumptions. Based on these somewhat conflicting data, some associations do seem plausible.

2.4 Microsatellite instability (MSI) and CpG island methylator phenotype (CIMP) in colorectal cancer

2.4.1 MSI and CIMP as genomic instabilities in colorectal cancer

Acquired variation in length of repetitive DNA sequences (microsatellites) can be detected as microsatellite instability (MSI) and is prevalent in approximately 15% of sporadic CRC and in almost all CRC in Lynch/Hereditary Non-Polyposis Colorectal Cancer syndrome (Soreide et al., 2006). MSI arises as a result of DNA replication errors that produce a change in length of repetitive sequences, which if not repaired (by the DNA mismatch repair (MMR) system), accumulate with increasing frequency (Martin et al., 2010; Soreide et al., 2006). In sporadic CRC, the most frequent inactivating cause of MMR is the methylation of the *MLH1* promoter on one or both alleles (Herman et al., 1998; Wheeler et al., 2000).

The MMR process is responsible for the correction of DNA replication errors which result in small insertions or deletions in the genome; these are especially prevalent at microsatellites due to increased frequency of DNA polymerase slippage at repetitive sequences. In humans, two major components comprise the MMR pathway: MutS (which is present in two heterodimers of MSH2/MSH6 and MSH2/MSH3) and MutL (which is also present in several heterodimer forms:- MLH1/PMS2, MLH1/PMS1 and MLH1/MLH3) (Martin et al., 2010). Disruption of the formation of the MutS and MutL dimers (by abrogation of the component proteins due to acquired promoter methylation or mutation) leads to a limited or defective MMR pathway, giving rise to genomic instability whereby DNA regions, most frequently repetitive sequences, increase or decrease in length (MSI). Such instability can lead to gene mutations, frequently of frameshift type, which can contribute to cancer progression.

CIMP is observed in 30-40% of proximal colonic and 3-12% of distal/rectal cancers (Curtin et al., 2011; Ibrahim et al., 2011). The exact causes of excessive methylation in DNA regions harbouring high levels of adjacent cytosine and guanine bases (CpG islands) are

unknown, although some evidence exists which suggest that such an increase in methyl group incorporation at these sites occurs during ageing in normal epithelial cells in the gut, and this is elevated in cancer (Toyota et al., 1999). Hypermethylation of gene promotors, in addition to or independent of methylation of other local DNA sequences, leads to transcriptional silencing of those genes. Such transcriptional silencing can be considered as one mechanism by which genes can be 'knocked out', in addition to mutation and deletion, in Knudson's model of tumour suppressor gene inactivation (Kondo & Issa, 2004). In this way, the aberrant methylation of genes can contribute to their inactivation in cancer epigenetically, such that in the absence of inactivating genetic changes tumour suppressor gene activity can be lost, leading to cancer progression.

2.4.2 MSI and dietary associations

MSI in CRC has been assessed in relation to dietary intakes in several reports, many of which did not identify a link between this type of genomic instability in CRC and specific dietary intakes (Chang et al., 2007; de Vogel et al., 2008; Jensen et al., 2008; Schernhammer, Giovannuccci, Fuchs & Ogino, 2008) (Table 2). However, a limited number of studies have described links between dietary intakes and MSI in colorectal neoplasms. An analysis of 144 microsatellite stable (MSS) and 40 MSI colonic cancers described an increased intake of red meat as associated with increased prevalence of MSS cancers (Diergaarde, Braam, van Muijen, Ligtenberg, Kok & Kampman, 2003). However, an assessment of 437 MSS and 49 MSI colonic cancers, failed to identify a similar association with red meat and MSS status (Satia et al., 2005). Additionally, a further report, testing 238 MSS and 35 MSI colonic cancers also failed to identify red meat intake as associated with MSI or MSS status (Wu et al., 2001). However, in the study performed by Wu and colleagues, heterocyclic amines were found to be associated with increased prevalence of MSI CRC. Heterocyclic amines can be produced during certain high-temperature methods of cooking of meats (Santarelli et al., 2008). Consequently, it is plausible that cooking method, independent of, or in conjunction with, certain meat types, may be associated with MSI status in CRC, potentially explaining the inconsistent observations between MSI and meat intakes.

Alcohol intake has been described as associated with MSI status in CRC. One report, analysing 1337 MSS and 227 MSI CRC identified increased alcohol intake as associated with a higher prevalence of MSS cancers (Poynter et al., 2009). Discordantly, a second analysis of 1244 MSS and 266 MSI colonic cancers identified increased alcohol consumption as linked to increased prevalence of MSI cancers (Slattery et al., 2001).

Folate intake has also been assessed relative to MSI status in CRC. Increased levels of plasma folate were associated with MSI cancer prevalence in a report assessing 166 MSS and 24 MSI CRC (Van Guelpen et al., 2010). However, assessment of dietary intake of folate in studies testing 179 MSS and 16 MSI CRC (Chang et al., 2007), 572 MSS and 76 MSI CRC (de Vogel et al., 2008), 111 MSS and 19 MSI CRC (Jensen et al., 2008) and 542 MSS and 127 MSI colonic cancers (Schernhammer, Giovannuccci, Fuchs & Ogino, 2008) all identified no association between folate intake and MSI status in CRC.

Presently, the data describing dietary associations and MSI status in CRC are contradictory and difficult to interpret. No strong associations have been identified and corroborated in independent cohorts. The difficulty in identification of plausible dietary constituents which may affect MSI prevalence in CRC may be due to the lack of such a relationship existing. It

Study	MSS/MSI-low CRC/CC	MSI/MSI-high CRC/CC	dietary association
Chang *et al* 2007	CRC: 179	CRC: 16	no statistically significant association between folate or vitamin B12 and MSI status
de Vogel *et al* 2008	CRC: 572	CRC: 76	no statistically significant association between folate, vitamin B2, methionine or alcohol and MSI status
Diergaarde *et al* 2003	CC: 144	CC: 40	↑ red meat associated with MSS cancers
Jensen *et al* 2008	CRC: 111	CRC: 19	no association between MSI and folate or vitamin B12
Poynter *et al* 2009	CRC: 1337	CRC: 227	↑ alcohol associated with MSS cancers
Satia *et al* 2005	CC: 437	CC: 49	no association between diet and MSI status [some associations comparing MSI/MSS cases vs controls]
Schernhammer *et al* 2008	CC: 542	CC: 127	no statistically significant association between folate, vitamin B6, B12, methionine or alcohol and MSI status
Slattery *et al* 2001	CC: 1244	CC: 266	↑ alcohol associated with MSI cancers
Van Guelpen *et al* 2010	CRC: 166	CRC: 24	Increased levels of plasma folate associated with MSI cancers
Wu *et al* 2001	CC: 238	CC: 35	↑ heterocyclic aromatic amines associated with MSI cancers

Table 2. Summarised description of literature analysing microsatellite instability (MSI) in colorectal neoplasia in relation to dietary intakes with the statistically significant associations described. *MSS*: microsatellite stability, *WT*: wildtype, *CRC*: colorectal cancer, *CC*: colonic cancer, ↑ and ↓ denote an increase or decrease in consumption respectively.

may also be plausible that such relationships exist and are particularly subtle. Methylation of the *MLH1* promoter, leading to gene silencing and subsequent DNA MMR deficiency, occurs in the vast majority, but not all, of MSI CRC (Kuismanen et al., 2000); suggesting that other components of the MMR system can be disrupted, such as mutations to the *MSH2* or *MSH6* genes, and that MSI may develop from a group of distinct initial aberrations in a small proportion of CRC. Furthermore, subsequent instability at microsatellites as a result may depend on other promoting factors. As such, it appears that a series of molecular events takes place leading to the MSI phenotype, which may arise from different epigenetic silencing or mutational events in different cancers. The multiple causes of MSI, and the different associated factors, may explain, at least in part, the lack of consistently identified dietary constituents which have been associated with this type of genomic instability. Alternatively, age-related susceptibility to promoter methylation, including the MLH1 promoter, may be the predominant risk factor for MSI in CRC rather than dietary factors.

2.4.3 CIMP and dietary associations

Studies assessing dietary associations with CIMP in CRC have centred largely on testing intakes of those compounds which may act as methyl group donors, or which function in the biochemical pathways responsible for methylation processes. Vitamin B6 has been described as associated with an increased prevalence of CIMP in CRC in one study assessing 496 CIMP-low/absent and 152 CIMP-high cancers (de Vogel et al., 2008). However, several other reports, assessing 288 CIMP-low/absent and 87 CIMP-high (Schernhammer et al., 2011) and 824 CIMP-low/absent and 330 CIMP-high (Slattery et al., 2007) colonic cancers failed to identify a similar association.

A similar lack of consensus has been observed when assessing vitamin B12. A single study assessing 107 CIMP-low/absent and 44 CIMP-high colonic cancers described an increased serum vitamin B12 concentration as associated with CIMP in this cohort (Mokarram et al., 2008). Schernhammer and colleagues (Schernhammer et al., 2011) and Slattery and co-workers (Slattery et al., 2007) did not identify a similar association in their studies. A report assessing 163 CIMP-low/absent and 27 CIMP-high CRC also identified no association between vitamin B12 intakes and CIMP status (Van Guelpen et al., 2010). Assessment of folate intake in relation to CIMP status has consistently failed to identify associations between the two in both colorectal and colonic cancer studies (Schernhammer et al., 2011; Slattery et al., 2007; Van Guelpen et al., 2010).

A single report, assessing 167 CIMP-low/negative and 17 CIMP-high colonic cancers identified reduced fruit intake as associated with an increased prevalence of CIMP-high colonic cancer (Diergaarde, Braam, van Muijen, Ligtenberg, Kok & Kampman, 2003). In an independent study, reduced consumption of vitamin A was identified as associated with increased prevalence of CIMP-high CRC (98 CIMP-low/absent CRC and 22 CIMP-high CRC) (Mas et al., 2007). These observations are yet to be corroborated in other studies. An additional report, assessing 776 CIMP-low/absent and 74 CIMP-high rectal cancers (Slattery, Curtin, Wolff, Herrick, Caan & Samowitz, 2010) failed to identify fruit intakes as associated with CIMP-high rectal cancer prevalence. Little additional data exists describing vitamin A intakes relative to CIMP status in CRC.

A limited number of additional associations have been observed relating CIMP status to certain dietary patterns. One report, assessing broad dietary patterns in addition to specific nutrient and foodstuff intakes identified increased fat-rich dairy products and omega-3 fatty acid consumption as associated with increased frequency of CIMP-high rectal cancers (776 CIMP-low/absent cancers and 74 CIMP-high cancers) (Slattery, Curtin, Wolff, Herrick, Caan & Samowitz, 2010). In an additional analysis, using this same patient cohort, long-term liquor/spirit intake was also found to be associated with an increased prevalence of CIMP-high status (Slattery, Wolff, Herrick, Curtin, Caan & Samowitz, 2010). Very few studies have assessed alcohol intake in terms of beverage consumed, as such, this observation awaits confirmation in an independent study. Additional data do not exist at present which validate the observed associations between increased consumption of fat-rich dairy products and omega-3 fatty acid with CIMP-high status.

There is no dietary intake which has been identified in several cohorts as associated with CIMP-high colorectal neoplasia. This may be due to the variety of methodologies used to assess CIMP status and the different criteria used to define CIMP-high status in these cancers, with no consensus method and definition having been used across studies (see Table 3). Furthermore, CIMP itself is the resulting phenotype of precursor genetic and epigenetic aberrations. As such, it may be plausible that this CRC subtype may not be linked to dietary risk factors, but instead diet may be linked to the causative precursor events, such as *MLH1* promoter methylation and MSI. Assessment of large study cohorts, in which CIMP-high cancers are categorised by causative lesions or processes, would in part help to understand dietary intakes and causation in the context of this phenotype.

3. Review limitations

This review has attempted to assess the available data describing the relationship between dietary factors and the molecular genetic events occurring during the development and progression of CRC. Published analyses have been summarised and where consensus between studies exists, this has been highlighted. Although providing a synopsis of the available information, several limitations are inherent in such a general discussion.

No detailed analysis or discussion of the methods of statistical analysis in each report has been provided. The wide range of methodology employed for this purpose across studies makes such a discussion in the present chapter impractical. Opinions on statistical methods vary across reports in terms of adjustment for multiple testing, inclusion of confounding variables in statistical models and the requirement for power calculations. In this context, no discussion or comparison of statistical methods has been attempted; notably, hazard and odds

Study	CIMP-low/absent CRC/CC/RC	CIMP-high CRC/CC/RC	dietary association
de Vogel *et al* 2008	CRC: 496*	CRC: 152*	↑ vitamin B6 associated with *MLH1* promoter methylation in males only
Diergaarde *et al* 2003	CC: 167**	CC: 17**	↓ fruit associated with *MLH1* promoter methylation and concurrent absence of MLH1 protein
Mas *et al* 2007	CRC: 98*	CRC: 22*	↓ vitamin A associated with *MLH1* promoter methylation
Mokarram *et al* 2008	CC: 107∓	CC: 44∓	increased levels of serum B12 associated with CIMP
Schernhammer *et al* 2011	CC: 288†	CC: 87†	no association between folate, vitamin B6, B12, methionine or alcohol consumption and CIMP
Slattery *et al* 2007	CC: 824††	CC: 330††	no association between folate, vitamin B6, B12, methionine or alcohol consumption and CIMP
Slattery *et al* 2010	RC: 776††	RC: 74††	no association between calcium and vitamin D consumption and CIMP
Slattery *et al* 2010	RC: 776††	RC: 74††	↑ fat-rich dairy products and ↑ omega-3 fatty acids associated with CIMP
Slattery *et al* 2010	RC: 776††	RC: 74††	long-term ↑ spirits/liquor with CIMP
Van Guelpen *et al* 2010	CRC: 163‡	CRC: 27‡	no association between plasma folate or plasma vitamin B12 and CIMP

Table 3. Summarised description of literature analysing CpG island methylator phenotype (CIMP) in colorectal neoplasia in relation to dietary intakes with the statistically significant associations described. *WT*: wildtype, *CRC*: colorectal cancer, *CC*: colonic cancer, *RC*: rectal cancer, ↑ and ↓ denote an increase or decrease in consumption respectively. * CIMP positive status defined by *MLH1* promoter methylation. ** CIMP positive status defined by *MLH1* promoter methylation and concurrent loss of MLH1 expression as determined by immunohistochemistry. ∓ CIMP positive status defined by methylation of one or more of the *p16*, *MLH1* or *MSH2* promoters. † CIMP positive status defined by methylation at 11 of 16 tested markers. †† CIMP positive status defined by methylation at 2 of 5 tested markers. ‡ CIMP positive status defined by methylation at 6 of 8 tested markers.

ratios should be further analysed in order to interpret the relative 'strength' of the associations highlighted here.

In addition to statistical methods, the methodology of dietary assessment in each individual report has not been discussed. Dietary intakes can be measured in a variety of ways, including person-to-person interview, food frequency questionnaires, food diaries and biomarker assessment. Such an assessment is beyond the scope of this chapter. Outside of this review, several specific reports have been published describing the merits, limits and practicality of some of the available options for dietary assessment (Bingham et al., 1995; Day et al., 2001). To fully interpret dietary associations identified in different studies, although not discussed herein, an appreciation of dietary assessment methodology, and the relative accuracy of such techniques, should be taken into account.

Further to the limits inherent in the compilation of this review, consideration of the nature of assessment of dietary intakes relative to characteristics of colorectal cancers is required. For example, considerably more data exist describing the relationship between mutations in *K-RAS* and diet than for *APC*. An 'assessment bias' exists, presumably due to the significantly simpler task of examining hotspot mutation regions of the *K-RAS* proto-oncogene compared with the longer lengths of sequencing required for mutational assessment of tumour suppressor genes. As a result, the molecular genetic changes which occur during CRC development have not been assessed at equal frequencies. Such 'assessment bias' should be noted when considering such a broad view, as presented in this chapter. This should be particularly considered when trying to interpret the genetic or molecular changes which have been tested in relation to diet in only a small number of studies.

It should also be understood that in many reports assessing dietary associations in CRC broad definitions are employed, in order to maintain the practicality and feasibility of studies. For example, often reports describing mutations in *BRAF* are actually describing mutations only in exons 11 and 15; reports describing *p53* mutations are frequently only describing mutational analyses of exons 5-8. Such limited analyses of coding regions is justified, with the significant majority of mutations in these examples being present in the regions described.

Furthermore, such limitations increase the practicality of these studies, in terms of both financial support and time investments required. Additionally, these limited regions of analyses are frequently selected based on biological evidence. Although justified, the limited extent to which genes are searched for the presence of mutations should be appreciated, and such variability between studies may in part explain inconsistent observations. In conjunction with this, different methods of mutational assessment provide different levels of sensitivity. For example, hotspot mutational assessment has been demonstrated to be more sensitively performed using pyrosequencing compared with dideoxysequencing (Naguib et al., 2010; Ogino et al., 2005). Such discrepancies between different reports were not discussed in this chapter, but should be considered when making side-by-side comparisons of studies.

In addition to the genetic and epigenetic changes giving rise to CRC development and progression described in this chapter, additional events occur during progression of these neoplasms. Furthermore, these events may be associated with dietary intakes, and data exist describing their associations with dietary consumptions; for example, loss of PTEN expression has also been tested for association with dietary intakes in CRC (Naguib, Cooke, Happerfield, Kerr, Gay, Luben, Ball, Mitrou, McTaggart & Arends, 2011). Studies of genetic and epigentic events beyond those discussed here were omitted due to the current low number of studies assessing their relationship with diet.

4. Future directions of the field

Next generation sequencing technology now affords the practical and accurate sequencing of entire genomes, with such strategies being employed to assess the genetic changes in several cancer types (Stratton et al., 2009). Furthermore, genomewide single nucleotide polymorphism analyses are being employed in a variety of settings. With these tools it is now possible to ask different questions relating diet to cancer. Are certain chemicals in the diet associated with an increased prevalence of any type of base change across the genome? Are transitions or transversions associated with intakes of specific compounds? The prospect of such investigations greatly expand the potential to understand the biochemical implications of certain dietary intakes, and provide an attractive avenue by which the identification of initiating factors in colorectal carcinogenesis might be pursued.

At present, a moderate number of studies have attempted to assess what impact, if any, dietary factors may have on CRC and the molecular subtypes of tumours which comprise this disease. With new technologies becoming available which have the power to expand this field of study, the underlying question of the purpose of such analyses should be clarified. Simply identifying dietary links to disease is only of limited use: how can this understanding be employed to reduce cancer-related mortality? It may be unrealistic to expect that if dietary constituents can be shown to be associated with increased prevalence of any particular molecular subtypes of colorectal cancer that these may be eliminated from the diet. The overwhelming evidence describing the strong association between tobacco use and cancer mortality fails to deter a significant number of smokers; although, the identification of such a link has undoubtedly provided individuals with knowledge upon which informed decisions have been made to refrain from tobacco use. Instead, a more 'protective' approach might be endorsed, such that dietary constituents which are found to confer protection against certain types of CRC might be promoted. This may be particularly useful in the attempt to lower the number of diagnoses of the molecular subtypes of CRC which confer a poor

prognosis. Some have suggested that excessive administration of dietary advice may prove to be counterproductive: advice should be administered sparsely and where the greatest potential to reduce cancer-related deaths exists. It is in this context that the understanding of diet and the molecular subtypes of CRC has the greatest potential and will provide the greatest impact in the effort to reduce the number of CRC-related deaths.

5. Conclusions and summary

At present, although data exist describing the association of particular dietary factors with the specific molecular genetic changes in CRC, very few consistently reproducible associations have been described. Several factors may contribute to this, including variations in study methodologies (dietary intake assessment, sequencing strategies), statistical assessment (variation in the statistical power/number of samples, inclusion of different confounding variables in models) and features of study design.

Assessment of the presently available data do describe some associations which warrant further study: *K-RAS* mutation appears to be less frequent in CRC in individuals consuming a high folate diet. Furthermore, *APC* mutation appears to be associated with meat intakes to some degree, although this exact relationship is unclear.

At present, the study of diet in relation to the specific subtypes of CRC is at an exciting stage. Sequencing technology advancements now provide an avenue by which the total genetic composition of CRC, and the specific molecular subtypes, can be assessed. Using such tools, detailed understanding of genomewide events can be correlated with dietary intakes. Such modern approaches, coupled with renewed efforts to improve, validate and employ the most reliable and accurate methods of dietary intake assessment, provide the keys to the success of this field, which will help to provide the sought after end goal of a reduction in the number of CRC-related deaths.

6. Acknowledgments

This work was supported by EPIC Norfolk and the Medical Research Council Centre for Nutritional Epidemiology in Cancer Prevention and Survival. Furthermore, the dedication and support of Professor Kay-Tee Khaw and the late Professor Sheila Bingham were essential in the completion of this chapter.

7. References

Arends, M. J., McGregor, A. H., Toft, N. J., Brown, E. J. & Wyllie, A. H. (1993). Susceptibility to apoptosis is differentially regulated by c-myc and mutated Ha-ras oncogenes and is associated with endonuclease availability, *Br J Cancer* 68(6): 1127–1133.

Arends, M. J., McGregor, A. H. & Wyllie, A. H. (1994). Apoptosis is inversely related to necrosis and determines net growth in tumors bearing constitutively expressed myc, ras, and HPV oncogenes, *Am J Pathol* 144(5): 1045–1057.

Aylon, Y. & Oren, M. (2011). New plays in the p53 theater, *Curr Opin Genet Dev* 21(1): 86–92.

Bautista, D., Obrador, A., Moreno, V., Cabeza, E., Canet, R., Benito, E., Bosch, X. & Costa, J. (1997). Ki-ras mutation modifies the protective effect of dietary monounsaturated

fat and calcium on sporadic colorectal cancer, *Cancer Epidemiol Biomarkers Prev* 6(1): 57–61.

Bingham, S. A., Cassidy, A., Cole, T. J., Welch, A., Runswick, S. A., Black, A. E., Thurnham, D., Bates, C., Khaw, K. T. & Key, T. J. (1995). Validation of weighed records and other methods of dietary assessment using the 24 h urine nitrogen technique and other biological markers, *Br J Nutr* 73(4): 531–550.

Bongaerts, B. W. C., de Goeij, A. F. P. M., van den Brandt, P. A. & Weijenberg, M. P. (2006). Alcohol and the risk of colon and rectal cancer with mutations in the K-ras gene, *Alcohol* 38(3): 147–154.

Bos, J. L. (1989). Ras oncogenes in human cancer: a review., *Cancer Res* 49(17): 4682–4689.

Bourdon, J. C., Laurenzi, V. D., Melino, G. & Lane, D. (2003). p53: 25 years of research and more questions to answer., *Cell Death Differ* 10(4): 397–399.

Boyle, P. & Langman, J. S. (2000). ABC of colorectal cancer: Epidemiology., *BMJ* 321(7264): 805–808.

Brink, M., Weijenberg, M. P., de Goeij, A. F. P. M., Roemen, G. M. J. M., Lentjes, M. H. F. M., de Bruïne, A. P., Goldbohm, R. A. & van den Brandt, P. A. (2005). Meat consumption and K-ras mutations in sporadic colon and rectal cancer in The Netherlands Cohort Study, *Br J Cancer* 92(7): 1310–1320.

Brink, M., Weijenberg, M. P., de Goeij, A. F. P. M., Roemen, G. M. J. M., Lentjes, M. H. F. M., de Bruïne, A. P., van Engeland, M., Goldbohm, R. A. & van den Brandt, P. A. (2005). Dietary folate intake and k-ras mutations in sporadic colon and rectal cancer in The Netherlands Cohort Study, *Int J Cancer* 114(5): 824–830.

Brink, M., Weijenberg, M. P., De Goeij, A. F. P. M., Schouten, L. J., Koedijk, F. D. H., Roemen, G. M. J. M., Lentjes, M. H. F. M., De Bruïne, A. P., Goldbohm, R. A. & Van Den Brandt, P. A. (2004). Fat and K-ras mutations in sporadic colorectal cancer in The Netherlands Cohort Study, *Carcinogenesis* 25(9): 1619–1628.

Ceol, C. J., Pellman, D. & Zon, L. I. (2007). APC and colon cancer: two hits for one., *Nat Med* 13(11): 1286–1287.

Chang, S.-C., Lin, P.-C., Lin, J.-K., Yang, S.-H., Wang, H.-S. & Li, A. F.-Y. (2007). Role of MTHFR polymorphisms and folate levels in different phenotypes of sporadic colorectal cancers, *Int J Colorectal Dis* 22(5): 483–489.

Curtin, K., Slattery, M. L. & Samowitz, W. S. (2011). CpG island methylation in colorectal cancer: past, present and future, *Patholog Res Int* 2011: 902674.

Day, N., McKeown, N., Wong, M., Welch, A. & Bingham, S. (2001). Epidemiological assessment of diet: a comparison of a 7-day diary with a food frequency questionnaire using urinary markers of nitrogen, potassium and sodium, *Int J Epidemiol* 30(2): 309–317.

de Vogel, S., Bongaerts, B. W. C., Wouters, K. A. D., Kester, A. D. M., Schouten, L. J., de Goeij, A. F. P. M., de Bruïne, A. P., Goldbohm, R. A., van den Brandt, P. A., van Engeland, M. & Weijenberg, M. P. (2008). Associations of dietary methyl donor intake with MLH1 promoter hypermethylation and related molecular phenotypes in sporadic colorectal cancer, *Carcinogenesis* 29(9): 1765–1773.

de Vogel, S., van Engeland, M., Lüchtenborg, M., de Bruïne, A. P., Roemen, G. M. J. M., Lentjes, M. H. F. M., Goldbohm, R. A., van den Brandt, P. A., de Goeij, A. F. P. M.

& Weijenberg, M. P. (2006). Dietary folate and APC mutations in sporadic colorectal cancer, *J Nutr* 136(12): 3015–3021.

Diergaarde, B., Braam, H., van Muijen, G. N. P., Ligtenberg, M. J. L., Kok, F. J. & Kampman, E. (2003). Dietary factors and microsatellite instability in sporadic colon carcinomas, *Cancer Epidemiol Biomarkers Prev* 12(11 Pt 1): 1130–1136.

Diergaarde, B., Tiemersma, E. W., Braam, H., van Muijen, G. N. P., Nagengast, F. M., Kok, F. J. & Kampman, E. (2005). Dietary factors and truncating APC mutations in sporadic colorectal adenomas, *Int J Cancer* 113(1): 126–132.

Diergaarde, B., van Geloof, W. L., van Muijen, G. N. P., Kok, F. J. & Kampman, E. (2003). Dietary factors and the occurrence of truncating APC mutations in sporadic colon carcinomas: a Dutch population-based study, *Carcinogenesis* 24(2): 283–290.

Eichholzer, M., Luthy, J., Moser, U. & Fowler, B. (2001). Folate and the risk of colorectal, breast and cervix cancer: the epidemiological evidence., *Swiss Med Wkly* 131(37-38): 539–549.

Fodde, R., Smits, R. & Clevers, H. (2001). APC, signal transduction and genetic instability in colorectal cancer., *Nat Rev Cancer* 1(1): 55–67.

Forbes, S. A., Bhamra, G., Bamford, S., Dawson, E., Kok, C., Clements, J., Menzies, A., Teague, J. W., Futreal, P. A. & Stratton, M. R. (2008). The Catalogue of Somatic Mutations in Cancer (COSMIC), *Curr Protoc Hum Genet* Chapter 10: Unit 10.11.

Fransen, K., Klintenas, M., Osterstrom, A., Dimberg, J., Monstein, H.-J. & Soderkvist, P. (2004). Mutation analysis of the BRAF, ARAF and RAF-1 genes in human colorectal adenocarcinomas., *Carcinogenesis* 25(4): 527–533.

Freedman, A. N., Michalek, A. M., Marshall, J. R., Mettlin, C. J., Petrelli, N. J., Black, J. D., Zhang, Z. F., Satchidanand, S. & Asirwatham, J. E. (1996). Familial and nutritional risk factors for p53 overexpression in colorectal cancer, *Cancer Epidemiol Biomarkers Prev* 5(4): 285–291.

Freudenheim, J. L., Graham, S., Marshall, J. R., Haughey, B. P., Cholewinski, S. & Wilkinson, G. (1991). Folate intake and carcinogenesis of the colon and rectum., *Int J Epidemiol* 20(2): 368–374.

Goss, K. H. & Groden, J. (2000). Biology of the adenomatous polyposis coli tumor suppressor, *J Clin Oncol* 18(9): 1967–1979.

Herman, J. G., Umar, A., Polyak, K., Graff, J. R., Ahuja, N., Issa, J. P., Markowitz, S., Willson, J. K., Hamilton, S. R., Kinzler, K. W., Kane, M. F., Kolodner, R. D., Vogelstein, B., Kunkel, T. A. & Baylin, S. B. (1998). Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma, *Proc Natl Acad Sci U S A* 95(12): 6870–6875.

Ibrahim, A. E. K., Arends, M. J., Silva, A.-L., Wyllie, A. H., Greger, L., Ito, Y., Vowler, S. L., Huang, T. H.-M., Tavaré, S., Murrell, A. & Brenton, J. D. (2011). Sequential DNA methylation changes are associated with DNMT3B overexpression in colorectal neoplastic progression, *Gut* 60(4): 499–508.

Jen, J., Powell, S. M., Papadopoulos, N., Smith, K. J., Hamilton, S. R., Vogelstein, B. & Kinzler, K. W. (1994). Molecular determinants of dysplasia in colorectal lesions., *Cancer Res* 54(21): 5523–5526.

Jensen, L. H., Lindebjerg, J., Crüger, D. G., Brandslund, I., Jakobsen, A., Kolvraa, S. & Nielsen, J. N. (2008). Microsatellite instability and the association with plasma homocysteine and thymidylate synthase in colorectal cancer, *Cancer Invest* 26(6): 583–589.

Kambara, T., Simms, L. A., Whitehall, V. L., Spring, K. J., Wynter, C. V., Walsh, M. D., Barker, M. A., Arnold, S., McGivern, A., Matsubara, N., Tanaka, N., Higuchi, T., Young, J., Jass, J. R. & Leggett, B. A. (2004). BRAF mutation is associated with DNA methylation in serrated polyps and cancers of the colorectum, *Gut* 53(8): 1137–1144.

Kim, Y.-I. (2005). Nutritional epigenetics: impact of folate deficiency on DNA methylation and colon cancer susceptibility, *J Nutr* 135(11): 2703–2709.

Kinzler, K. W. & Vogelstein, B. (1996). Lessons from hereditary colorectal cancer, *Cell* 87(2): 159–170.

Kondo, Y. & Issa, J.-P. J. (2004). Epigenetic changes in colorectal cancer, *Cancer Metastasis Rev* 23(1-2): 29–39.

Kuhnle, G. G. & Bingham, S. A. (2007). Dietary meat, endogenous nitrosation and colorectal cancer, *Biochem Soc Trans* 35(Pt 5): 1355–1357.

Kuhnle, G. G. C., Story, G. W., Reda, T., Mani, A. R., Moore, K. P., Lunn, J. C. & Bingham, S. A. (2007). Diet-induced endogenous formation of nitroso compounds in the GI tract., *Free Radic Biol Med* 43(7): 1040–1047.

Kuismanen, S. A., Holmberg, M. T., Salovaara, R., de la Chapelle, A. & Peltomäki, P. (2000). Genetic and epigenetic modification of MLH1 accounts for a major share of microsatellite-unstable colorectal cancers, *Am J Pathol* 156(5): 1773–1779.

Laso, N., Mas, S., Jose Lafuente, M., Casterad, X., Trias, M., Ballesta, A., Molina, R., Salas, J., Ascaso, C., Zheng, S., Wiencke, J. K. & Lafuente, A. (2004). Decrease in specific micronutrient intake in colorectal cancer patients with tumors presenting Ki-ras mutation, *Anticancer Res* 24(3b): 2011–2020.

Lee, S., Cho, N. Y., Choi, M., Yoo, E. J., Kim, J. H. & Kang, G. H. (2008). Clinicopathological features of CpG island methylator phenotype-positive colorectal cancer and its adverse prognosis in relation to KRAS/BRAF mutation, *Pathol Int* 58(2): 104–113.

Lüchtenborg, M., Weijenberg, M. P., de Goeij, A. F. P. M., Wark, P. A., Brink, M., Roemen, G. M. J. M., Lentjes, M. H. F. M., de Bruïne, A. P., Goldbohm, R. A., van 't Veer, P. & van den Brandt, P. A. (2005). Meat and fish consumption, APC gene mutations and hMLH1 expression in colon and rectal cancer: a prospective cohort study (The Netherlands), *Cancer Causes Control* 16(9): 1041–1054.

Luo, F., Brooks, D. G., Ye, H., Hamoudi, R., Poulogiannis, G., Patek, C. E., Winton, D. J. & Arends, M. J. (2007). Conditional expression of mutated K-ras accelerates intestinal tumorigenesis in Msh2-deficient mice, *Oncogene* 26(30): 4415–4427.

Luo, F., Brooks, D. G., Ye, H., Hamoudi, R., Poulogiannis, G., Patek, C. E., Winton, D. J. & Arends, M. J. (2009). Mutated K-ras(Asp12) promotes tumourigenesis in Apc(Min) mice more in the large than the small intestines, with synergistic effects between K-ras and Wnt pathways, *Int J Exp Pathol* 90(5): 558–574.

Luo, F., Poulogiannis, G., Ye, H., Hamoudi, R. & Arends, M. J. (2011). Synergism between K-rasVal12 and mutant Apc accelerates murine large intestinal tumourigenesis, *Oncol Rep* 26(1): 125–133.

Luo, F., Poulogiannis, G., Ye, H., Hamoudi, R., Zhang, W., Dong, G. & Arends, M. J. (2011). Mutant K-ras promotes carcinogen-induced murine colorectal tumourigenesis, but does not alter tumour chromosome stability, *J Pathol* 223(3): 390–399.

Martin, S. A., Lord, C. J. & Ashworth, A. (2010). Therapeutic targeting of the DNA mismatch repair pathway, *Clin Cancer Res* 16(21): 5107–5113.

Martínez, M. E., Maltzman, T., Marshall, J. R., Einspahr, J., Reid, M. E., Sampliner, R., Ahnen, D. J., Hamilton, S. R. & Alberts, D. S. (1999). Risk factors for Ki-ras protooncogene mutation in sporadic colorectal adenomas, *Cancer Res* 59(20): 5181–5185.

Mas, S., Lafuente, M. J., Crescenti, A., Trias, M., Ballesta, A., Molina, R., Zheng, S., Wiencke, J. K. & Lafuente, A. (2007). Lower specific micronutrient intake in colorectal cancer patients with tumors presenting promoter hypermethylation in p16(INK4a), p4(ARF) and hMLH1, *Anticancer Res* 27(2): 1151–1156.

McMichael, A. J. & Giles, G. G. (1988). Cancer in migrants to Australia: extending the descriptive epidemiological data., *Cancer Res* 48(3): 751–756.

Melhem, M. F., Law, J. C., el Ashmawy, L., Johnson, J. T., Landreneau, R. J., Srivastava, S. & Whiteside, T. L. (1995). Assessment of sensitivity and specificity of immunohistochemical staining of p53 in lung and head and neck cancers, *Am J Pathol* 146(5): 1170–1177.

Mokarram, P., Naghibalhossaini, F., Saberi Firoozi, M., Hosseini, S. V., Izadpanah, A., Salahi, H., Malek-Hosseini, S. A., Talei, A. & Mojallal, M. (2008). Methylenetetrahydrofolate reductase C677T genotype affects promoter methylation of tumor-specific genes in sporadic colorectal cancer through an interaction with folate/vitamin B12 status, *World J Gastroenterol* 14(23): 3662–3671.

Naguib, A., Cooke, J. C., Happerfield, L., Kerr, L., Gay, L. J., Luben, R. N., Ball, R. Y., Mitrou, P. N., McTaggart, A. & Arends, M. J. (2011). Alterations in PTEN and PIK3CA in colorectal cancers in the EPIC Norfolk study: associations with clinicopathological and dietary factors, *BMC Cancer* 11: 123.

Naguib, A., Mitrou, P. N., Gay, L. J., Cooke, J. C., Luben, R. N., Ball, R. Y., McTaggart, A., Arends, M. J. & Rodwell, S. A. (2010). Dietary, lifestyle and clinicopathological factors associated with BRAF and K-ras mutations arising in distinct subsets of colorectal cancers in the EPIC Norfolk study, *BMC Cancer* 10: 99.

Naguib, A., Wilson, C. H., Adams, D. J. & Arends, M. J. (2011). Activation of K-RAS by co-mutation of codons 19 and 20 is transforming, *J Mol Signal* 6: 2.

Norat, T., Bingham, S., Ferrari, P., Slimani, N., Jenab, M., Mazuir, M., Overvad, K., Olsen, A., Tjonneland, A., Clavel, F., Boutron-Ruault, M.-C., Kesse, E., Boeing, H., Bergmann, M. M., Nieters, A., Linseisen, J., Trichopoulou, A., Trichopoulos, D., Tountas, Y., Berrino, F., Palli, D., Panico, S., Tumino, R., Vineis, P., Bueno-de Mesquita, H. B., Peeters, P. H. M., Engeset, D., Lund, E., Skeie, G., Ardanaz, E., Gonzalez, C., Navarro, C., Quiros, J. R., Sanchez, M.-J., Berglund, G., Mattisson, I., Hallmans, G., Palmqvist, R., Day, N. E., Khaw, K.-T., Key, T. J., San Joaquin, M., Hemon, B., Saracci, R., Kaaks, R. & Riboli, E. (2005). Meat, fish, and colorectal cancer risk: the European Prospective Investigation into cancer and nutrition., *J Natl Cancer Inst* 97(12): 906–916.

O'Brien, H., Matthew, J. A., Gee, J. M., Watson, M., Rhodes, M., Speakman, C. T., Stebbings, W. S., Kennedy, H. J. & Johnson, I. T. (2000). K-ras mutations, rectal crypt cells

proliferation, and meat consumption in patients with left-sided colorectal carcinoma, *Eur J Cancer Prev* 9(1): 41–47.

Ogino, S., Kawasaki, T., Brahmandam, M., Yan, L., Cantor, M., Namgyal, C., Mino-Kenudson, M., Lauwers, G. Y., Loda, M. & Fuchs, C. S. (2005). Sensitive sequencing method for KRAS mutation detection by Pyrosequencing., *J Mol Diagn* 7(3): 413–421.

Park, J. Y., Mitrou, P. N., Keen, J., Dahm, C. C., Gay, L. J., Luben, R. N., McTaggart, A., Khaw, K.-T., Ball, R. Y., Arends, M. J. & Rodwell, S. A. (2010). Lifestyle factors and p53 mutation patterns in colorectal cancer patients in the EPIC-Norfolk study, *Mutagenesis* 25(4): 351–358.

Parkin, D. M., Bray, F., Ferlay, J. & Pisani, P. (2005). Global cancer statistics, 2002., *CA Cancer J Clin* 55(2): 74–108.

Peyssonnaux, C. & Eychene, A. (2001). The Raf/MEK/ERK pathway: new concepts of activation, *Biol Cell* 93(1-2): 53–62.

Poulogiannis, G., Ichimura, K., Hamoudi, R. A., Luo, F., Leung, S. Y., Yuen, S. T., Harrison, D. J., Wyllie, A. H. & Arends, M. J. (2010). Prognostic relevance of DNA copy number changes in colorectal cancer, *J Pathol* 220(3): 338–347.

Poulogiannis, G., McIntyre, R. E., Dimitriadi, M., Apps, J. R., Wilson, C. H., Ichimura, K., Luo, F., Cantley, L. C., Wyllie, A. H., Adams, D. J. & Arends, M. J. (2010). PARK2 deletions occur frequently in sporadic colorectal cancer and accelerate adenoma development in Apc mutant mice, *Proc Natl Acad Sci U S A* 107(34): 15145–15150.

Poynter, J. N., Haile, R. W., Siegmund, K. D., Campbell, P. T., Figueiredo, J. C., Limburg, P., Young, J., Le Marchand, L., Potter, J. D., Cotterchio, M., Casey, G., Hopper, J. L., Jenkins, M. A., Thibodeau, S. N., Newcomb, P. A., Baron, J. A. & Colon Cancer Family Registry (2009). Associations between smoking, alcohol consumption, and colorectal cancer, overall and by tumor microsatellite instability status, *Cancer Epidemiol Biomarkers Prev* 18(10): 2745–2750.

Robinson, M. J. & Cobb, M. H. (1997). Mitogen-activated protein kinase pathways, *Curr Opin Cell Biol* 9(2): 180–186.

Samowitz, W. S., Albertsen, H., Herrick, J., Levin, T. R., Sweeney, C., Murtaugh, M. A., Wolff, R. K. & Slattery, M. L. (2005). Evaluation of a large, population-based sample supports a CpG island methylator phenotype in colon cancer, *Gastroenterology* 129(3): 837–845.

Santarelli, R. L., Pierre, F. & Corpet, D. E. (2008). Processed meat and colorectal cancer: a review of epidemiologic and experimental evidence, *Nutr Cancer* 60(2): 131–144.

Satia, J. A., Keku, T., Galanko, J. A., Martin, C., Doctolero, R. T., Tajima, A., Sandler, R. S. & Carethers, J. M. (2005). Diet, lifestyle, and genomic instability in the North Carolina Colon Cancer Study, *Cancer Epidemiol Biomarkers Prev* 14(2): 429–436.

Schernhammer, E. S., Giovannuccci, E., Fuchs, C. S. & Ogino, S. (2008). A prospective study of dietary folate and vitamin B and colon cancer according to microsatellite instability and KRAS mutational status, *Cancer Epidemiol Biomarkers Prev* 17(10): 2895–2898.

Schernhammer, E. S., Giovannucci, E., Baba, Y., Fuchs, C. S. & Ogino, S. (2011). B vitamins, methionine and alcohol intake and risk of colon cancer in relation to BRAF mutation and CpG island methylator phenotype (CIMP), *PLoS One* 6(6): e21102.

Schernhammer, E. S., Ogino, S. & Fuchs, C. S. (2008). Folate and vitamin B6 intake and risk of colon cancer in relation to p53 expression, *Gastroenterology* 135(3): 770–780.

Sjöblom, T., Jones, S., Wood, L. D., Parsons, D. W., Lin, J., Barber, T. D., Mandelker, D., Leary, R. J., Ptak, J., Silliman, N., Szabo, S., Buckhaults, P., Farrell, C., Meeh, P., Markowitz, S. D., Willis, J., Dawson, D., Willson, J. K. V., Gazdar, A. F., Hartigan, J., Wu, L., Liu, C., Parmigiani, G., Park, B. H., Bachman, K. E., Papadopoulos, N., Vogelstein, B., Kinzler, K. W. & Velculescu, V. E. (2006). The consensus coding sequences of human breast and colorectal cancers, *Science* 314(5797): 268–274.

Slattery, M. L., Anderson, K., Curtin, K., Ma, K. N., Schaffer, D. & Samowitz, W. (2001). Dietary intake and microsatellite instability in colon tumors, *Int J Cancer* 93(4): 601–607.

Slattery, M. L., Curtin, K., Anderson, K., Ma, K. N., Edwards, S., Leppert, M., Potter, J., Schaffer, D. & Samowitz, W. S. (2000). Associations between dietary intake and Ki-ras mutations in colon tumors: a population-based study, *Cancer Res* 60(24): 6935–6941.

Slattery, M. L., Curtin, K., Ma, K., Edwards, S., Schaffer, D., Anderson, K. & Samowitz, W. (2002). Diet activity, and lifestyle associations with p53 mutations in colon tumors., *Cancer Epidemiol Biomarkers Prev* 11(6): 541–548.

Slattery, M. L., Curtin, K., Sweeney, C., Levin, T. R., Potter, J., Wolff, R. K., Albertsen, H. & Samowitz, W. S. (2007). Diet and lifestyle factor associations with CpG island methylator phenotype and BRAF mutations in colon cancer, *Int J Cancer* 120(3): 656–663.

Slattery, M. L., Curtin, K., Wolff, R. K., Herrick, J. S., Caan, B. J. & Samowitz, W. (2010). Diet, physical activity, and body size associations with rectal tumor mutations and epigenetic changes, *Cancer Causes Control* 21(8): 1237–1245.

Slattery, M. L., Wolff, R. K., Herrick, J. S., Curtin, K., Caan, B. J. & Samowitz, W. (2010). Alcohol consumption and rectal tumor mutations and epigenetic changes, *Dis Colon Rectum* 53(8): 1182–1189.

Soreide, K., Janssen, E. A., Soiland, H., Korner, H. & Baak, J. P. (2006). Microsatellite instability in colorectal cancer, *Br J Surg* 93(4): 395–406.

Stratton, M. R., Campbell, P. J. & Futreal, P. A. (2009). The cancer genome, *Nature* 458(7239): 719–724.

Suehiro, Y. & Hinoda, Y. (2008). Genetic and epigenetic changes in aberrant crypt foci and serrated polyps., *Cancer Sci* 99(6): 1071–1076.

Takayama, T., Katsuki, S., Takahashi, Y., Ohi, M., Nojiri, S., Sakamaki, S., Kato, J., Kogawa, K., Miyake, H. & Niitsu, Y. (1998). Aberrant crypt foci of the colon as precursors of adenoma and cancer., *N Engl J Med* 339(18): 1277–1284.

Toyota, M., Ahuja, N., Ohe-Toyota, M., Herman, J. G., Baylin, S. B. & Issa, J. P. (1999). CpG island methylator phenotype in colorectal cancer, *Proc Natl Acad Sci U S A* 96(15): 8681–8686.

Van Guelpen, B., Dahlin, A. M., Hultdin, J., Eklöf, V., Johansson, I., Henriksson, M. L., Cullman, I., Hallmans, G. & Palmqvist, R. (2010). One-carbon metabolism and CpG island methylator phenotype status in incident colorectal cancer: a nested case-referent study, *Cancer Causes Control* 21(4): 557–566.

Velho, S., Moutinho, C., Cirnes, L., Albuquerque, C., Hamelin, R., Schmitt, F., Carneiro, F., Oliveira, C. & Seruca, R. (2008). BRAF, KRAS and PIK3CA mutations in colorectal serrated polyps and cancer: primary or secondary genetic events in colorectal carcinogenesis?, *BMC Cancer* 8: 255.

Voskuil, D. W., Kampman, E., van Kraats, A. A., Balder, H. F., van Muijen, G. N., Goldbohm, R. A. & van't Veer, P. (1999). p53 over-expression and p53 mutations in colon carcinomas: relation to dietary risk factors, *Int J Cancer* 81(5): 675–681.

Wark, P. A., Van der Kuil, W., Ploemacher, J., Van Muijen, G. N. P., Mulder, C. J. J., Weijenberg, M. P., Kok, F. J. & Kampman, E. (2006). Diet, lifestyle and risk of K-ras mutation-positive and -negative colorectal adenomas, *Int J Cancer* 119(2): 398–405.

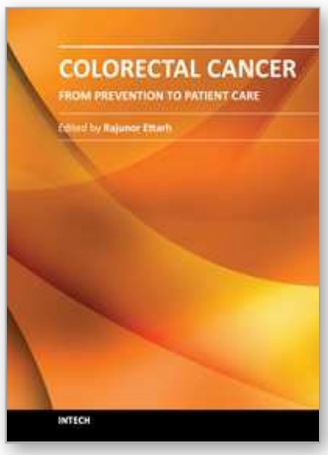
Weijenberg, M. P., Luchtenborg, M., de Goeij, A. F., Brink, M., van Muijen, G. N., de Bruine, A. P., Goldbohm, R. A. & van den Brandt, P. A. (2007). Dietary fat and risk of colon and rectal cancer with aberrant MLH1 expression, APC or KRAS genes, *Cancer Causes Control* 18(8): 865–879.

Wheeler, J. M., Loukola, A., Aaltonen, L. A., Mortensen, N. J. & Bodmer, W. F. (2000). The role of hypermethylation of the hMLH1 promoter region in HNPCC versus MSI+ sporadic colorectal cancers, *J Med Genet* 37(8): 588–592.

Wood, L. D., Parsons, D. W., Jones, S., Lin, J., Sjoblom, T., Leary, R. J., Shen, D., Boca, S. M., Barber, T., Ptak, J., Silliman, N., Szabo, S., Dezso, Z., Ustyanksky, V., Nikolskaya, T., Nikolsky, Y., Karchin, R., Wilson, P. A., Kaminker, J. S., Zhang, Z., Croshaw, R., Willis, J., Dawson, D., Shipitsin, M., Willson, J. K., Sukumar, S., Polyak, K., Park, B. H., Pethiyagoda, C. L., Pant, P. V., Ballinger, D. G., Sparks, A. B., Hartigan, J., Smith, D. R., Suh, E., Papadopoulos, N., Buckhaults, P., Markowitz, S. D., Parmigiani, G., Kinzler, K. W., Velculescu, V. E. & Vogelstein, B. (2007). The genomic landscapes of human breast and colorectal cancers, *Science* 318(5853): 1108–1113.

Wu, A. H., Shibata, D., Yu, M. C., Lai, M. Y. & Ross, R. K. (2001). Dietary heterocyclic amines and microsatellite instability in colon adenocarcinomas, *Carcinogenesis* 22(10): 1681–1684.

Zhang, Z. F., Zeng, Z. S., Sarkis, A. S., Klimstra, D. S., Charytonowicz, E., Pollack, D., Vena, J., Guillem, J., Marshall, J. R., Cordon-Cardo, C., Cohen, A. M. & Begg, C. B. (1995). Family history of cancer, body weight, and p53 nuclear overexpression in Duke's C colorectal cancer, *Br J Cancer* 71(4): 888–893.



Colorectal Cancer - From Prevention to Patient Care
Edited by Dr. Rajunor Ettarh

ISBN 978-953-51-0028-7
Hard cover, 538 pages
Publisher InTech
Published online 17, February, 2012
Published in print edition February, 2012

The projections for future growth in the number of new patients with colorectal cancer in most parts of the world remain unfavorable. When we consider the substantial morbidity and mortality that accompanies the disease, the acute need for improvements and better solutions in patient care becomes evident. This volume, organized in five sections, represents a synopsis of the significant efforts from scientists, clinicians and investigators towards finding improvements in different patient care aspects including nutrition, diagnostic approaches, treatment strategies with the addition of some novel therapeutic approaches, and prevention. For scientists involved in investigations that explore fundamental cellular events in colorectal cancer, this volume provides a framework for translational integration of cell biological and clinical information. Clinicians as well as other healthcare professionals involved in patient management for colorectal cancer will find this volume useful.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen