Effect of food components on colon cancer through microbial transformation in the colon

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Abstract

Because the mortality rates for colorectal cancer are high, it is of importance that research is done so that risks can be reduced. A major risk factor involved in the development of colon cancer is the composition of the diet. Various strains of bacteria can act tumor promoting or can act against the risk of developing cancer. A close interaction exists between food substances and the microbiota, as the microbiota can be modulated by food compounds and microbiotic enzymes can convert food compounds into other substances. Carbohydrates can be fermented to short-chain fatty acids (SCFAs) which act preventive, while proteolytic fermentation can lead to an increase in toxic substances that can act on the progression of colorectal cancer. In future, research can be further focused on mechanisms behind these phenomena.

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1. Colon cancer

1.1 Prevalence

Colon cancer is one of the leading causes of death through malignancies in developed countries nowadays (Satia-Abouta, 2003). The mortality rates for colon cancer are amongst the highest for cancer, along with lung cancer and breast cancer. Mortality rates around the globe were around 600.000 in 2008, which is 8.2% of the total mortality due to cancer (IARC, 2010). In the past 20-30 years there was little indication of decrease in mortality rates (Collins, Rafter et al., 2007). Furthermore, the incidence for colon cancer doubled to 1.2 million, which is 17.2% of the total cancer incidence (IARC, 2010). The 5 year survival from colon cancer varies demographically, and is estimated to be 65% in North America, 54% in Western Europe, 34% in Eastern Europe and 30% in India (Parkin et al, 2002).

1.2 Risk factors and epidemiology with a focus on food

The main risk factors that are involved with the development of colon cancer are a low physical activity, obesity and the diet composition. This literature review will be further focused on the composition of the diet as a risk factor. What is already known

is that composition of the diet contributes to the risk for colon cancer (Collins, 2007). Dietary behavior such as the intake of a high-fat and low-fiber diet, are major contributors involved in the risk (Niamsup, Thirabunyanon et al., 2009). Furthermore, in epidemiological studies it was observed that the intake of vegetables, fruits and other plant products are associated with a reduction in risk (McKeown-Eyssen, 1994). Other life style factors associated with a decrease in the risk for colon cancer are postmenopausal hormone use, non-steroidal inflammatory drug use, high calcium intake, exercise and the use of fish oil. While smoking cigarettes, consumption of alcohol, obesity and physical inactivity are associated with an increase in risk (Potter, 1999).

In general it can be stated that life style behavior involved in high serum triglycerides and plasma glucose concentrations are associated with an increased risk for the development of colon cancer (McKewon-Eyssen, 1994). This can be affirmed by case-control and cohort studies, in which was found that energy-containing nutrients, fat, protein and simple carbohydrates are risk factors in the development of colorectal cancer. Whereas the consumption of vegetables, fruits and cereals showed a decrease in risk, something that might be due to components in plant food, such as fiber and vitamins (McKeown-Eyssen, 1994).

1.3 Biology

Colon cancer is a disease originating from the epithelium that is lining the colon and rectum. The rate in which the epithelial cells replicate is relatively high, 10^{10} cells are replaced every day (Komarova, 2005). The risk for colon cancer is increasing with age, because multiple mutations are needed to develop this disease (Alberts, 2003). However, in a small part of the cases (15%), the family is prone to colon cancer due to mutation with an autosomal-dominant fashion in which there can be an unusual early onset. In the other 85% of the cases, no genetic predisposition is present as far as is currently known.

Involved in the development of colon cancer is the *Wnt*-signalling pathway (*Figure 1*), as this pathway is involved in the stimulation of cell proliferation in the crypts that are lining the gut. The pathway can be disrupted as a result of successive genetic mutations. Because multiple mutations are needed to develop colon cancer, most patients are of older age. Genes in the *Wnt*-pathway that are frequently mutated in colon cancer are *K-Ras*, *p53* and *APC*. *K-Ras* is an oncogene, in 40% of the colon cancer cases point mutations are observed in this gene. In 60% of the cases inactivating mutations or deletions are observed in *p53*. And in over 60% of the tumors both copies of the *APC* gene are lost; while in adjacent tissue still two copies of the gene are present. *APC* is a tumor suppressor gene. When the *APC* gene is lost, the *Wnt* pathway will become hyperactive which will result in an excessive proliferation of cells. This neoplastic growth is called a polyp which is a precursor for colon cancer. The polyps can develop into an invasive cancer because further mutations may occur (Alberts, 2003). To demonstrate the effect of various dietary components on the development of colon cancer, expression levels of the various genes involved in the *Wnt* pathway can be measured (Kumar, 2010).

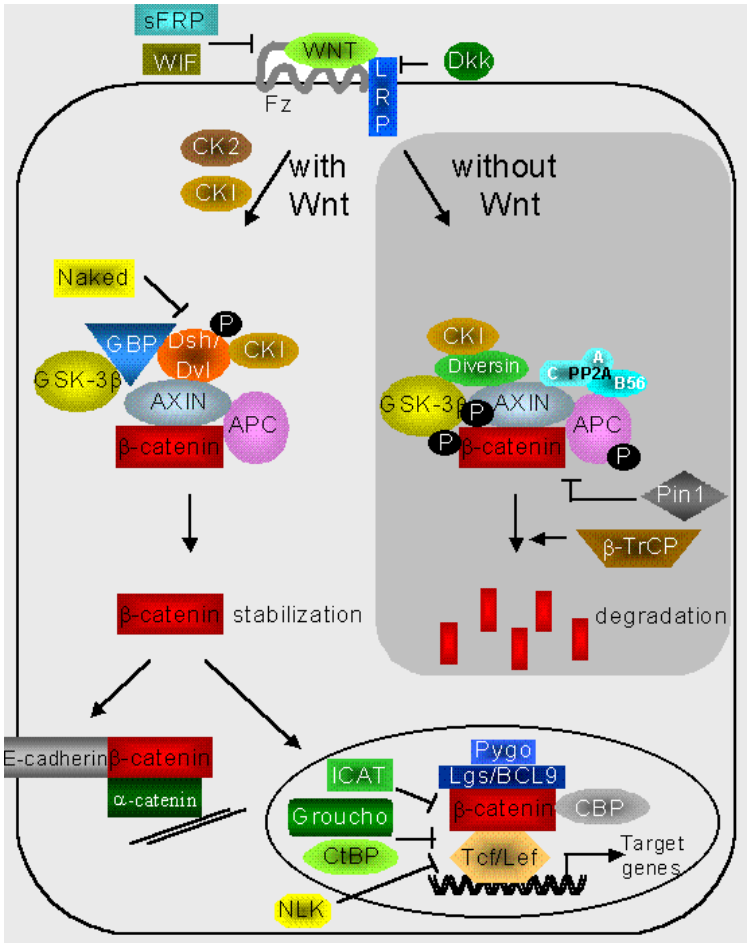

Figure 1: Wnt signalling pathway, schematic presentation (Thorstensen, 2003)

2. Microbiota in the colon

2.1 Genera and species

The human adult gut contains about 100 trillion microbial organisms, which as a total is called the microbiota (Davis, 2009). This microbiota normally contains over 500 different bacterial species, of which the highest concentration is found in the colon (Guarner, 2006). The human microbiota is dominated by strict anaerobe bacteria; with genera that include *Bacteriodes*, *Eubacterium*, *Fusobacterium*, *Peptostreptococcus* and *Atpobium* (Davis, 2009). In numbers that lay around 1000-fold lower, facultative anaerobes are present such as *Lactobacillus*, *Enterococcus*, and *Enterobacteriaceae*. Genera or species that might be involved in the pathogenesis of cancer include *Streptococcus bovis*, *Bacteriodes*, *Clostridia* and

Helicobacter pylori, while species that include *Lactobacillus acidophilus* and *Bifidobacterium longum* are shown to inhibit tumor development induced by carcinogens (Davis, 2009). The composition of the microbiota in the gut can be modified by the diet (Sarbini, 2011).

2.2. Mechanism of action

The microbiota can be involved in the development of colon cancer, as well in the prevention as in the onset. Mechanisms that are involved are for instance production of substances from dietary components that have genotoxic, carcinogenic and tumor-promoting activities (Niamsup, 2009). The release of carcinogens in the intestinal tract can be the result of metabolic actions of bacterial enzymes. Enzymes produced by the microbiota can transform dietary components into tumor-promoting, genotoxic and carcinogenic substances. It was found that bacterial enzymes present in the feces are associated with the conversion of pro-carcinogens to carcinogens (Karthik kumar, 2009). Enzymes that are associated with the transformation of pre-carcinogens into carcinogens are for instance β-glucuronidase, nitroreductase, azoreductase, 7-α-dehydroxylase and cholesterol dehydrogenase (Niamsup, 2009). The β-glucuronidase enzyme is known to be involved in the generation of toxic and carcinogenic metabolites by hydrolyzing glucuronide conjugates (Karthik kumar, 2008). These glucuronides usually are synthesized by the liver as a detoxification mechanism and the glucuronides are excreted from the body in the bile. When the bile reached the intestines, the glucuronides cannot be absorbed in the small intestine and thus reach the colon. Here, the bacterial β-glucuronidase can cleave of the glucoside from the detoxified glucuonide, thus forming the original toxic compound again. This compound (unless further metabolized by the microbiota) can interact with the epithelial layer, which are the first cells of the body it gets into contact with, and influence colon cancer onset or progression.

Two examples of bacterial species that can act tumor promoting are *Streptococcus bovis* and *Helicobacter pylori*. *Streptococcus bovis* can be found in the normal microbiota of the gastrointestinal (GI) tract in 5-16% of the adults (Noble, 1978). It is a Gram positive bacterium that can cause infections of endocarditis and bacteraemia. In many studies, it is linked with colon polyps and carcinomas (Burnett-Hartman, 2008). In this association several mechanisms have been hypothesized. One hypothesis is that the neoplastic sites in the colon can form niches for *Streptococcus bovis* so that survival of this bacterium is allowed and an infection is due to the local tumor. A second hypothesis is that an infection with *Streptococcus bovis* might promote the development of carcinogenesis. This last hypothesis is supported by several *in vivo* studies. Furthermore, an increase in interleukin IL-8 was found in this studies. IL-8 is a cytokine that plays a role in colon cancer as it has mitogenic and angiogenic properties (Tjalsma, 2012).

Helicobacter pylori is a bacterium that is found in approximately half of the world's population and is seen as an infection (Burnett-Hartman, 2008). It is a Gram-negative bacterium and is well adapted to the conditions in the stomach. The development of gastric carcinogenesis through this bacterium is thought to involve inflammation and the deregulation of the cell cycle. This deregulation occurs by a

Helicobacter pylori protein, which is encoded by the cytotoxin-associated gene A (*cagA*). CagA binds to SHP2, and activates this onco-protein what results in cell growth and motility (Lochhead, 2007). However, the relation between *Helicobacter pylori* and colorectal cancer is not yet clarified (Burnett-Hartman, 2008). Burnett-Hartman et al. found that in 16 epidemiological studies that examined the associations between colorectal cancer and *Helicobacter pylori*, six studies found statistically significant associations. Differences in the results in these studies might be due to differences in strains, as it was indicated that *Helicobacter pylori* $CagA^+$ strains are more likely to cause malignancy and inflammation than $CagA^-$ strains (Beales, 1996; Maeda, 2007).

On the other hand, there are also bacteria present in the microbiota which produce enzymes that can act preventive. Examples of these enzymes are β-galactosidase and α-glucosidase, which exert activities that can improve carbohydrate fermentation to short chain fatty acids (SCFAs) (Macfarlane, 1991). Strains from the genus *Bifidobacterium* are an example of bacteria exerting α-glucosidase enzyme activity (Sarbini, 2011). But also other mechanisms in which the microbiota can have a protecting potential for colon cancer are suggested. This can be due to mechanisms as alteration of the metabolic activities of intestinal microbiota and the physico-chemical condition in the colon, the production of anti-carcinogenic compounds, the enhancement of the immune response of the host and effects on physiology of the host (Niamsup, 2009). Mechanisms involved might include the reduction of the activity of cancer-causing bacteria, anti-mutagenic and anti-carcinogenic properties (Chau, 2006). Microbiota in the colon degrade undigested polysaccharides to SCFA's, synthesize biotin, foliate and vitamin K, ferment indigestible dietary residue, and assist in calcium, magnesium and iron absorption (Nowak, 2009).

Which bacterial species are exactly associated with colon cancer is not totally clarified yet, but known is that types as *Lactobacillus* and *Bifidobacterium* have a lower carcinogen producing enzyme activity than types as *Clostridium* and *Bacteroides*. Therefore, the balance in microbial strains in the gut might be of importance in the risk for colon cancer (Davis, 2009).

3. Food components that affect the risk for colon cancer

3.1 Carbohydrates vs. proteins

Food compounds that are not completely digested and absorbed in the small intestine can be fermented by the microbiota in the large intestine. The main types of anaerobic fermentation in the gastrointestinal tract are carbohydrate and proteolytic (Davis, 2009). It is thought that the main end product of carbohydrate metabolism reduce the cancer risk while end products associated with proteins might increase the risk. Carbohydrates can be fermented by bacteria so that SCFAs are produced. Examples of genera involved in this are *Bifidobacterium* and *Lactobacillus*, but many

more species are capable of doing this. *Bifidobacterium* and *Lactobacillus* are genera that produce the SCFAs acetate and lactate from out carbohydrates. These are intermediate products that can be further metabolized to butyrate and propionate by other bacteria such as *Eubacterium halii* and *Faecalibacterium prausnitzii*. Mainly butyrate is a SCFA that reduces the risk for colon cancer (Beards, 2010). SCFAs can lower the pH in the gut and may inhibit pathogens in the GI tract and this also can reduce the risk for colorectal cancer (Gibson, 1999). End products of proteolytic fermentation include phenolic compounds, such as indole, skatol and p-cresol and nitrogen-containing metabolites such as amines, ammonia, and *N*-nitroso compounds which can all be toxic (Davis, 2009).

3.2 Preventive potential of food compounds

Because the diet can change the composition of the microbiota in the gut, the diet can contribute to the enhancement of a beneficial microbiota in the gut. Food compounds that exert beneficial effects due to selective stimulation of growth or activity of gut microbiota are called prebiotics (Davis, 2009). Prebiotics exist of dietary carbohydrates that are not digested in the upper GI tract and alter the bacterial composition of the gut and can be an energy source for the microbiota (Gupta, 2009). It was found that a large number of dietary fiber fractions can act as prebiotics. These contain fructo-oligosaccharides (FOS), inulin, galacto-oligosaccharides (GOS) which can be derived from lactose, trans-galacto-oligosaccharides (TOS), and iso-malto-oligosaccharides (IMO) (Fooks, 1999; Blaut, 2002; Rastall, 2002). Members of the microbiota in the gut known for health promoting activities are *Bifidobacterium* and *Lactobacillus* (Beards, 2010). Therefore food components that increase the amount of these species are called prebiotics.

Probiotics are live microbial food ingredients that when ingested in adequate amounts have beneficial health effects (Salminen, 1998). An ideal probiotic is considered to be safe, resistant to bile, hydrochloric acid and pancreatic juice, has anti-carcinogenic activity and stimulates the immune system. It has reduced intestinal permeability, produces lactic acid and is able to survive the acidic conditions of the stomach and the alkaline conditions of the duodenum (Vimala, 2006). The most common types of microbes used as probiotics are *Bifidobacterium* and Lactic Acid Bacteria (LAB), which includes *Lactobacillus*. Foods that mainly contain LAB are fermented milks, cheeses, sausages, wine and fruit juices (Gupta, 2009). These bacteria can exert beneficial effects in the gastrointestinal (GI) tract. LAB can reduce levels of colon enzymes that convert pro-carcinogens to carcinogens. It mainly reduces enzyme levels of β-glucuronidase, nitro-reductase and azo-reducatse. Changes in enzyme activity in humans have been observed with *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (Marteau, et al. 1990), and *Lactobacillus rhamnosus GG* (LGG) (Goldin, 1984). Also, beneficial effects can be attributed to immune-potentiating effects by LAB strains (Karthik kumar, 2009). It has for instance been shown that probiotic bacteria modulate the immunoglobulin production. For example the probiotic LGG, has been proven to beneficially affect the intestinal immunity by increasing immunoglobulin secreting cells in the intestinal mucosa. (For example IgA is higher). In this way, LGG can stimulate the local release of interferons and it facilitates antigen transport to underlying lymphoid cells

(Reid, 2003). Probiotic bacteria can also increase profiles of cytokines TNF-α, IFN-γ, and IL-10. *Lactobacillus* and *Bifidobacterium* species have been frequently shown to modify the activity of the microbiota in the gut so that there is a reduction in β-glucuronidase and carcinogen levels (Reid, 2003; Ling 1994). Furthermore, it was found that lactobacilli might prevent or delay tumors in the intestine by binding to mutagenic compounds and suppressing the growth of bacteria that convert pro-carcinogens into carcinogens. The synergy between probiotics and prebiotics leads to the development of food products known which are known as synbiotic products (Roberfroid, 1998; Ziemer, 1998).

3.3 Carbohydrates

As eluded to above, from the fermentation of carbohydrates, such as GOS, FOS and inulin, SCFAs are produced (Davis, 2009). SCFAs are byproducts in the microbiotic fermentation of dietary fibers (Blottiere, 2003). In an *in vitro* study on the fermentation of dietary fiber of rice, it was shown that fermentation by pure cultures of probiotics lead to a significant increase in SCFA levels of acetate, butyrate and propionate (Fernando, 2010). Other metabolites that can be formed as a result of this fermentation are CO_2, H_2, CH_4, and other organic acids (Fernando, 2010). Examples of bacteria that ferment carbohydrates and produce acetate and lactate as main end product are *Lactobacillus* spp. and *Bifidobacterium* spp. (Zani, 1974). The interaction between prebiotics and probiotics also leads to the continuous production of SCFA.

In research performed *in vitro* as well as *in vivo*, it was found that SCFAs can regulate cell proliferation. The most important SCFA is butyrate, because it is the major source of energy for colonic epithelial cells and regulates cell growth and differentiation (Blottiere, 2003).Cell proliferation can be influenced by the release of growth factors or gastrointestinal peptides, or through modulation of mucosal blood flow. SCFA can act directly on genes that regulate cell proliferation, in which butyrate is the SCFA which shows most effect (Blottiere, 2003). At first, it was thought that effects that were observed seemed to be paradoxical. *In vivo* a trophic effect on the intestinal mucosa was observed; while *in vitro* an inhibitory effect on colonic neoplastic cells is found. However, it thus seems that the role in normal colonic cells is opposite to that of the role SCFA exert in neoplastic cells (Blottiere, 2003).

SCFAs in general and butyrate in particular are the main energy source for colonic epithelial cells. This means that cell proliferation is stimulated when this source of energy is compared with non-energetic/less-energetic substrates. Furthermore, it was shown that SCFAs are able to affect cell proliferation by the release of gastrointestinal peptides or growth factors. By acting on specific genes, butyrate can inhibit cell proliferation, stimulate differentiation and induce apoptosis. The mechanism of action of butyrate involves the induction of the expression of p21/Cipl at protein as well as at mRNA levels. This leads to an accumulation of cells in the G1 phase of the cell cycle, and therefore butyrate inhibits cell proliferation. Furthermore, it was found that cyclin D3 is up regulated. And that c-myc, which is the key transcription factor, is down regulated. Cdk inhibitor p21/Cipl has a structural role in

the butyrate-mediated G1 growth arrest, as it was observed that in absence of p21/Cipl, the cells enter the S phase (*Figure 2*) (Blottiere, 2003).

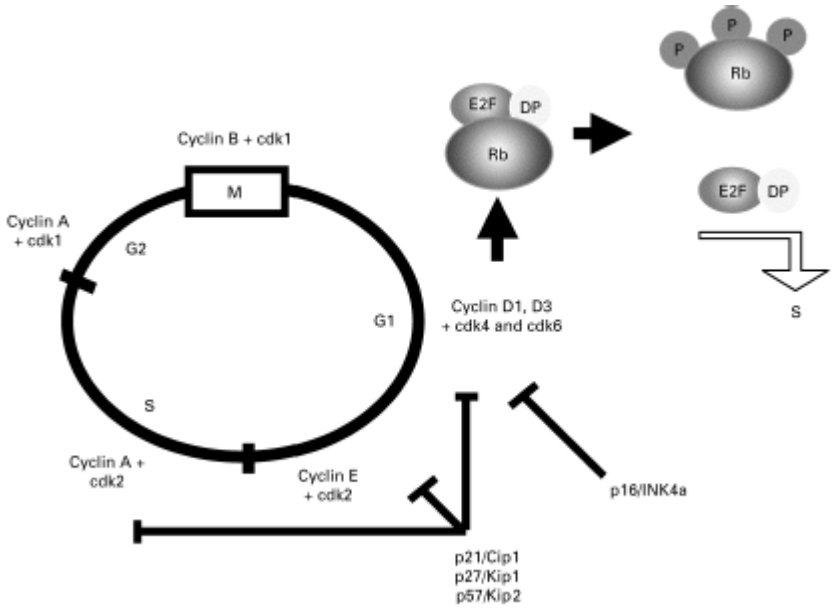

Figure 2: Proteins involved in cell proliferation. Butyrate can induce expression of p21/Cip1, so that cell proliferation is inhibited in the G1 phase of the cell cycle (Blottiere, 2003).

Butyrate has also been shown to hyperactivate the WNT/catenin signaling pathway, which has been shown to be important in colon cancer as eluded to above (Lazarova, 2004; Bordonaro, 2007). The hyperactivation of WNT/catenin signaling by butyrate takes place only in colonic neoplastic cells with mutations in the pathway, and such mutations are detected in 80% of the sporadic colon cancers.

3.4 Proteins

Proteolytic fermentation can result in the formation of substances that are toxic and can result in the formation of colon cancer. Examples of these substances are hydrogen sulfide, ammonia and phenolics. Further was found that bacterial populations can be modified as a result of diet. In an *in vivo* experiment in which mice were fed high-fat diets, a decrease in *Bacteroides*, *Eubacterium rectale/Clostridum coccoides* and *Bifidobacterium* was observed. What leads to an increase in endotoxemia resulting in diabetes (Cani, 2007). Moreover, the consumption of red meat can result in an increase in sulfate reducing bacteria, which produce hydrogen sulfide. Hydrogen sulfide is a genotoxic substance (Rooks, 2011). Proteins can also be a risk factor in the diet because preparation of meat at temperatures of 150-300 degrees can lead to the formation of heterocyclic aromatic

amines (HCA) out of amino acids. HCA are compounds that possess mutagenic potential and can contribute to the development of colorectal cancer (Nowak, 2009). Red meat can also increase the amounts of amines, nitrites and arginine's (Tjalsma, 2012). Furthermore, diets that are high in animal fats can stimulate growth of bacteria that produce secondary bile salt which is cytotoxic and carcinogenic (Nagengast 1995).

3.5 Other dietary components.

The intake of fiber (the major source of butyrate in the colon) is usually accompanied by that of other bioactive ingredients, and many fiber-rich foods are a source of polyphenols (e.g., cereals, fruit, and vegetables). The drinks that accompany our meals further increase the complexity of bioactives: wine, fruit juices, cocoa, tea, and coffee are also all rich in polyphenols. The two main classes of dietary polyphenols are the flavonoids and the phenolic acids. Similar to butyrate, polyphenols and their metabolites have been reported to exhibit a colon cancer protective role. For example, quercetin, a flavonol found in citrus fruit, buckwheat, and onions, suppresses the formation of aberrant crypt foci and induces apoptosis in preneoplastic human colonocytes (Herzog, 2004; Warren, 2009). Caffeic acid esters present in propolis are potent inhibitors of human colon adenocarcinoma cell growth, carcinogen-induced biochemical changes, and preneoplastic lesions in the rat colon (Rao, 1995; Rao, 1993). A colon cancer preventive role has also been reported for isoflavons, curcumin, and the major polyphenol in green tea, epigallocatechin-3-gallate (EGCG, Kumar, 2007).

Since the intake of dietary fiber is frequently accompanied by that of polyphenols, it was logical to test whether WNT/catenin signaling and apoptosis in colon cancer cells are modified by polyphenols and their (microbial) metabolites. Presently, the combined effects of butyrate and polyphenol metabolites on WNT/catenin signaling are unknown; however, there have been reports on the modulation of WNT/catenin signaling by polyphenols. For example, polymeric black tea polyphenols inhibit 1,2-dimethylhydrazine-induced colorectal tumorigenesis in rats, and it has been suggested that this effect is mediated by suppression of WNT/catenin signaling (Patel, 2008). EGCG suppresses WNT/catenin transcriptional activity in HCT-116 cells at concentrations of 100-200 µM, which are unachievable *in vivo* (Kim, 2006); however, at physiologically relevant concentration of 0.5 µM (Halliwell, 2005; Manach, 2005; Gao, 2006), EGCG inhibits the enzyme glycogen synthase kinase-3 beta (GSK-3beta, Pahlke, 2006). The inactivation of GSK-3beta should result in accumulation of the transcriptionally active Ser-37/Thr-41-dephosphorylated beta-catenin, the main mediator of WNT transcriptional activity (Staal, 2002; van Noort, 2002). Polyphenol-rich apple juice extract, as well as the free aglycon phloretin and the flavonol quercetin, also inhibit GSK-3beta in *in vitro* assays (Kern, 2006). In agreement with this inhibitory effect, quercetin increases WNT/catenin transcriptional activity by 15% at 10 µM, a concentration that also exceeds the levels achievable *in vivo* (Staal, 2002). The interpretation of these results is difficult due to the fact that the bioavailability of these compounds has not been taken into account, or is unknown. The inhibition of GSK-3beta by some polyphenols indicates that these compounds may synergize with butyrate in its effect on WNT/beta-catenin signaling.

Furthermore, similar to butyrate, some polyphenols and their metabolites inhibit histone deacetylases (HDACs). Thus, fermentation of polyphenol-rich apple juice extracts with human fecal slurry revealed that polyphenol metabolites have a HDAC inhibitory function (Waldecker, 2008). Metabolites of polyphenols in the colon, such as p-coumaric acid, 3-(4-OH-phenyl)-propionate, and caffeic acid also exhibit HDAC inhibitory function in *in vitro* assays with nuclear extracts from HT-29 human colon cancer cells (Waldecker, 2008). Therefore, similar to butyrate (Lazarova, 2004; Bordonaro, 2007), polyphenol metabolites with HDAC inhibitory function may protect against colon cancer via stabilization of beta-catenin and hyperinduction of WNT/beta-catenin signaling.

4. Discussion

In this literature review it is clarified that the diet can influence the risk for the onset and progression of colorectal cancer. Carbohydrates have a preventive potential through there conversion by the microbiota in protective metabolites such as butyrate and metabolites of polyphenols, while proteins can result in the formation of toxic end-products (Rooks, 2011). In future research the relationship between various types of bacteria and their role in colorectal cancer needs to be further clarified. As it is not yet totally clear which species exactly are involved in the development of cancer, although some are already determined. In addition, it should be further clarified what the specific role of these species is, such as for instance *Helicobacter pylori* (Burnett-Hartman, 2008). When mechanisms behind the involvement of the microbiota in colon cancer and the relation to the diet becomes clearer, food product can be developed that exert beneficial properties so that the risk for colorectal cancer can be reduced. Furthermore, the development of colorectal carcinogenesis can be prevented, by a reduction in the intake of food compounds that are positively associated with the risk for colon cancer.

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