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Review Article

Colon Cancer is Associated with Microbial Dysbiosis in Humans and Animals

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ABSTRACT

In Western countries there is a growing incidence in obesity, inflammatory bowel disease (IBD) and colorectal cancer (CRC), which places an increasingly important burden on the health care system. In the current decade, changes in intestinal microflora (i.e., dysbiosis) are likely the result of environment factors such as food, lifestyle, and medications which have been shown in obesity and IBD. In CRC, with the exception of germline DNA mutations which have been attributed to less than 5% of patients, little is still known about the main causes although the role of food is now suspected to have a major influence in the induction of cancer. Increasing data have shown specific changes in microflora in colon cancer patients' stools or adherent to the colonic mucosa, for which several mechanisms have been proposed using animal experiments. Thus, microbiota may be considered as a platform of host and environment interactions with which to study CRCs. Through new mechanisms in CRC pathophysiology including bacterial approach, the perspectives of screening, diagnostic and prognostic tests are discussed.

Keywords: Colon cancer; Bacteria; Genetic; Environment; Bacteria-host interaction

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INTRODUCTION

The incidence of colorectal cancer (CRC) is becoming a disease of high economic costs in developed countries. CRC has been steadily increasing over the last five decades; although the incidence is just beginning to be stabilized in some areas of the world, the mortality due to this disease remains elevated. A good understanding of the factors that promote carcinogenesis in CRC should enable a better policy of prevention and a more efficient screening protocol. The majority of CRC are considered to be sporadic, as they arise from environment rather than from germline mutations. Among environmental factors, a Western lifestyle is considered to favor the occurrence of CRC because developing countries have high incidences of CRC and they share a Western lifestyle.

Microbes have a major role in the biological environment; about 16% of cancers worldwide have been estimated to be caused by microbes. Cancers such as those arising from the liver and gastrointestinal tract are clearly identified as being microbe-related (1). Among these, *Helicobacter pylori* (*H. pylori*) are considered by the WHO Agency for Research on Cancer (IARC) to be associated with causation of gastric adenocarcinoma and MALT lymphoma. Evidences on such association have been obtained from epidemiological data in populations, experimental

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results in animal models and interventional trials of eradication of the bacterium in humans. *H. pylori* helped to better understanding of the physiology of the gastric function and new pathophysiology pathways in human gastric carcinogenesis.

In contrast, the microfloras of the colon comprise a very complex system that hosts several billions of bacteria with multitudes of functions. Due to the complexity of the current disease model used for colon carcinogenesis, it is not possible, at least at the present time, to conclusively link colonic carcinogenesis to a single bacterium or function. Here, we review recent data published within the last five years which supports the hypothesis that CRC is a bacteria-related disease. This will probably impact cancer growth and treatment response.

MICROBIOTA

Intestinal microbiota is a heterogeneous, complex entity composed of more than 1000 different bacterial species. In the colon, ~10^{12} bacteria exist in each mL, whereas less than ~10^{4} bacteria are found in the small intestine. Analysis of this microbiota makes it possible to study "factors from the environment" such as nutrients in addition to interactions with the host. This complex ecosystem contributes to the maturation of the immune system, provides a direct barrier against colonization of the intestine by pathogens(2) and appears to have the capability metabolize pro-carcinogens and carcinogens from the environment(3). Although more than 80% of intestinal bacteria cannot be cultured, bacterial identification has become possible by DNA analyses, which includes TTGE (Temporal Temperature Gel Electrophoresis) advanced technology to perform whole DNA genome sequencing. This metagenomic approach has allowed characterization of healthy individuals' microbiota(4) and is now considered as a reference. For more than ten years, DNA extractions from stools or colonic tissues have been regarded as a bar code and contributed to classify bacteria. Thus, attention has been focused on the observation that individual profiles display tremendous similarity and are partly significantly different (Figure 1). As a result, the bacterial community in an individual host is relatively stable in the distal digestive tract throughout the adult life, although much variation exists from person to person(5). The 'core microbiome' hypothesis, which states that a similar set of microbial functions can be found at the gene level in all individuals despite substantial variation at the species level may be a possible explanation for this phenomenon. Nevertheless, it seems plausible to expect that certain individuals (as determined by genetic make up and/or lifestyle) carry a higher proportion of bacterial drivers than other individuals and are therefore predisposed to bacteria-related diseases such as obesity, asthma, autoimmunity, or inflammatory bowel diseases (IBD)(6,7) and CRC. The main challenge is determining how to describe healthy conditions. Recent technological advances have yielded high levels of whole bacteria gene sequences. Despite large variations in healthy individuals' microflora (5), it has been shown that variations in intestinal microbiota are generally stratified rather than continuous. They might respond differently to diet and drug intake based on international analyses of healthy individuals' microbiota analyses(8). At least three well-defined enterotypes have been characterized from European healthy individuals' stools; such profiles could be similarly identified in the Japanese population. *Bacteroides* and *Prevotella* are two enterotypes that appear to be very discriminative according to the whole bacteria DNA sequence analyses (Figure 2). Further, data-driven marker genes or functional modules could be identified for host properties. For example, twelve genes that significantly correlate with age and three functional modules with the body mass index hint at the diagnostic potential of microbial markers(8).

After bacterial DNA extraction, temporal temperature gel electrophoresis called TTGE performed on 16S DNA after amplification shows bands that correspond to the molecular species. For each individual, profiles are equivalent to a fingerprint, showing high inter-individual variability. At the same time, samples that were collected over a two-year period from the same individual (1 to 3) show a high degree of similarity between the TTGE profiles as measured by the similarity between profiles on the dendrogram, with a similarity index above 92%.

The fecal microbiota of adults can be unique for each individual.

BACTERIA AND CRC LINKS

The numbers of bacteria in the large intestine is much greater than in the small intestine; this is paralleled by an approximately 12 fold increase in cancer risk for the large intestine compared to the small intestine. Together, these two observations point towards the hypothesis that colon cancer may be induced by bacteria.

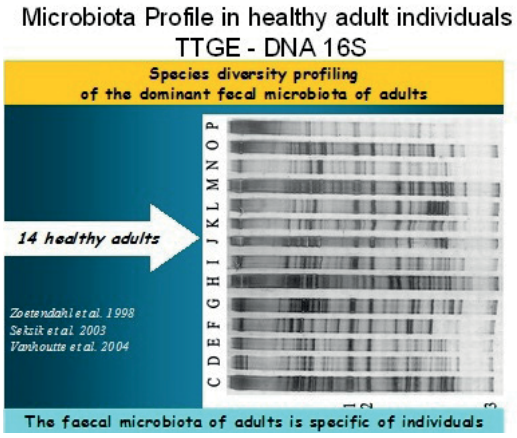

Fig. 1: **Profile of fecal microbiota**

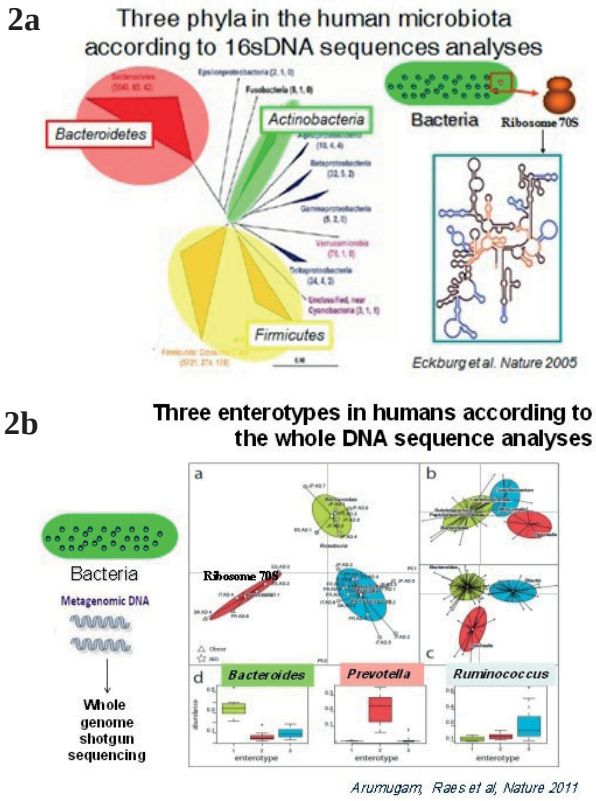

Fig. 2: **Bacteria clusters from human intestinal microbiota.**
2a subtitle: Three phyla in the human microbiota according to 16sDNA sequence analyses. Legends: After bacteria DNA extraction, the 16s DNA compound is amplified and submitted to the sequencing process and phylogenic patterns are estimated. Accordingly, three main dominant groups of bacteria are identified.2b subtitle: Enterotypes in humans according to whole DNA sequence analyses. After extraction of bacterial DNA, the DNA is submitted in its entirety to the sequencing process and bacteria species are estimated. Accordingly, three main enterotypes are identified. Figure 2b: Please change: "human" to "humans"; delete "the";"Genome" to "genome"; "Shotgun" to "shotgun". Italicize names of bacteria in picture.

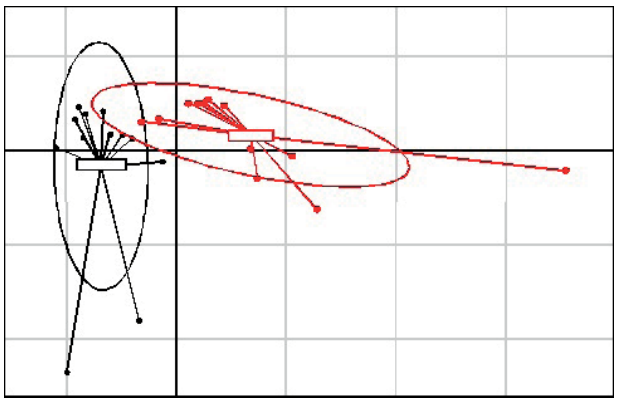

Fig. 3: **Composition of bacteria in colon cancer patients' stools.**
Legends: Fresh samples were collected prior to colonoscopy, DNA was extracted and submitted to pyrosequencing analyses of the 16sRNA V3-V4 region and comparisons of bacteria sequences were performed between individuals with normal colonoscopy results and colon cancer patients on the basis of abundance of bacterial species; this phylogenetic core permitted to distinguish cancer patients from healthy individuals. Principal component analysis, based on the 16S DNA gene sequence abundance that discriminated phylogenetic core species was performed with six normal individuals (red points) and six cancer patients (black points), with two replicates. The two first components (PC1 and PC2) were plotted, which represented 57.95% of whole inertia. Individuals (represented by their sample id) were clustered and the center of gravity computed for each class. This was the first comparison on stool composition in healthy individuals and in colon cancer patients that shows significant dysbiosis.

Historical data

Streptococcus bovis/gallolyticus (*S. bovis*) was traditionally considered to be a lower grade pathogen involved in endocarditis. Although McCoy and Mason(9) suggested a relationship between colonic carcinoma and the presence of infectious endocarditis in 1951, it was only in 1974 that the association of *S. bovis* and colorectal neoplasia was recognized.

In 1971, a study intended to identify associations between human microbiota composition and colorectal carcinogenesis without any specific hypothesis regarding a group of bacteria. This study was abandoned because of technical difficulties in recovering all bacteria from the stool samples, which included a large majority of anaerobic bacteria. Later, 13 bacterial species were shown to have a significant association with a high risk of colon cancer and the Western diet(10). However, these results were somewhat unconvincing because of the small number of subjects investigated, and the lack of an intestinal investigations such as radiology or colonoscopy studies.

Clinical data

The phylogenetic core characterized in healthy

individuals based on 16sDNA analyses, allowed comparison with diseased individual groups. Recently, we have reported that the phylogenic core of human microbiota significantly differed in colon cancer patients' stools (Figure 3) compared to matched normal colonoscopy individuals' stools; this result has been confirmed by others(11). Although these studies did not reveal whether phylogenetic core changes in colon cancer patients' stools were the cause or a consequence of the disease, quantification of major bacterial dominant and subdominant groups allowed identification of the *Bacteroides* family as the predominant bacteria in colon cancer patients. Interestingly, *Bacteroides fragilis*, a common intestinal commensal species from this bacteria family, has been shown to induce spontaneous colonic tumorigenesis in ApcMin/+ mice(12) compared to germ-free animals. Generally, in germ-free conditions, lower numbers of tumors are observed than when the conditions involve a conventional microbiota irrespective of whether the tumors have been induced by chemical agents or arose from germline mutations. However, the possibility that intestinal microorganisms have a direct effect on the initiation and progression of sporadic CRC has been ignored. A growing number of recent data support the hypothesis that CRC can be initiated by some 'driver' bacteria and promoted by 'passenger' bacteria(13). For bacteria to be a driver, adherence to the mucosa is presumed. However bacteria found in stools are not always detected among those attached to the epithelium and vice versa.

Mucosa-adherent bacteria

Epithelium-adherent bacteria appear to differ from stool *microbiota* and *Fusobacterium nucleatum* (*F. nucleatum*) in terms of abundance and heterogeneity. *F. nucleatum* is not a dominant species in stools; however two independent groups have detected it in tumor biopsies obtained from colon cancer patients (Figures 3 and 4). Possibly, this bacterium might be the cause of CRC(14,15), however it has not been prevalent in stool specimens examined in other studies(11,16). *F. nucleatum* is commonly found in the dental plaque of humans and is frequently associated with gum disease. It is a key component of periodontal plaque due to its abundance and its ability to coaggregate with other species in the oral cavity. The cells of *F. nucleatum* are found in the dental plaque of many primates, including humans. This bacterium displays the ability to associate with viruses that adhere to host tissue cells and modulate the host's immune response(17). However, a causative role of *Fusobacterium* is not demonstrated. Thus, it could be suggested that this bacterium is a co-factor of an unidentified cause. Currently, it is necessary to conduct studies on large numbers of patients regarding the abundance and heterogeneity of bacteria in normal and cancer tissues from the same individuals.

However, *Streptococcus gallyliticus* and *Fusobacterium* are probably passenger species. In pre-cancerous adenomatous polyps, studies of the 16S DNA genes from adherent bacteria have not shown that these two species are significant according to phylogenetic and taxonomic analyses(18,19). *Firmicutes* (62%), *Bacteroidetes* (26%) and *Proteobacteria* (11%) are the most dominant phyla among bacteria that adhere to pre-cancerous lesions. Although significant differences in bacterial composition between cases and controls have been identified, surprisingly in one of these studies(19) biopsies from adenomatous polyps showed a 20-fold relative reduction of mucosa adherent bacteria compared to normal tissue. The authors have proposed that adenoma mucosa might exert increased antibacterial activity compared to normal mucosa due to α-defensin production, which was significantly increased in the adenomatous polyps. To date no experimental data has supported this hypothesis.

Mechanisms of action of bacteria through colon carcinogenesis

In the original model of colon carcinogenesis it has been proposed(20) that only tubular and tubulovillous adenomas had the potential to progress to invasive adenocarcinoma. Serrated polyps, including sessile serrated adenomas (SSAs) and traditional serrated adenomas (TSAs), are recognized as having the potential for malignant transformation(21,22). A subset of hyperplastic polyps that are not usually recognized as precancerous lesions may progress to serrated neoplasms (SSAs or TSAs) and a fraction of these SSAs progress to cancer (23). In these histological lesions various alterations in genes such as APC, CTNNB1, DCC, P53, KRAS and MYC, or abnormalities such as chromosome instability, and microsatellite instability, and aberrant DNA methylation at CpG islands(22) are detected. These genetic alterations can be induced by chemical and/or environment factors. The occurrence of tumors is due to DNA alterations in the stem cells at the base of the crypt. These changes appear to be permanent and prone to the accumulation of additional mutations(24).

Stem cells as a pivotal target from normal cells to neoplastic lesions

Stem cells and their descendant proliferating crypt precursors(25) in addition to a large number of differentiated cells (enterocytes, enteroendocrine and goblet) occupy the crypt. These cells self-renew to regenerate the epithelium following injury(26-28). It is presumed that the first carcinogenic event occurs in stem and/or progenitor cells and CRC is considered to be the consequence of a succession of steps with identifiable specific gene mutations in cells(20). Here, the adherent bacteria could be considered as a crucial link with the environment, offering interactions with stem cells to yield focal lesions. In this regard the mucus layer(29) and crypt-specific core microbiota(30) are considered to be facilitators. In the two main hereditary syndromes that involve no lethal germline mutations (APC, MMR system) the delay for achieving a critical accumulation of mutations in stem cells up to the development of cancer is significantly shorter in adults (20 to 45 years of age) than in sporadic cancers (65 years of age).

The mosaic gene mutation, particularly in cases of lethal gene mutations is an exciting candidate to explain how accumulation of lethal gene mutations (Kras, beta cathenin or CTNNB1) are necessary at very early colon carcinogenic steps in lesions such as aberrant crypt foci (ACFs) or adenomas. For instance, a constitutional lethal gene mutation (c.49G-->A, p.Glu17Lys) in the oncogene AKT1 has recently been reported in tissues of patients with proteus syndrome, which is characterized by the overgrowth of skin. By using a custom restriction-enzyme test on DNA from patients with proteus syndrome, the authors have shown that this syndrome is caused by a lethal gene mutation through somatic mosaicism. The skin tissues harbor admixtures of mutant alleles that range from 1% to approximately 50% (31).

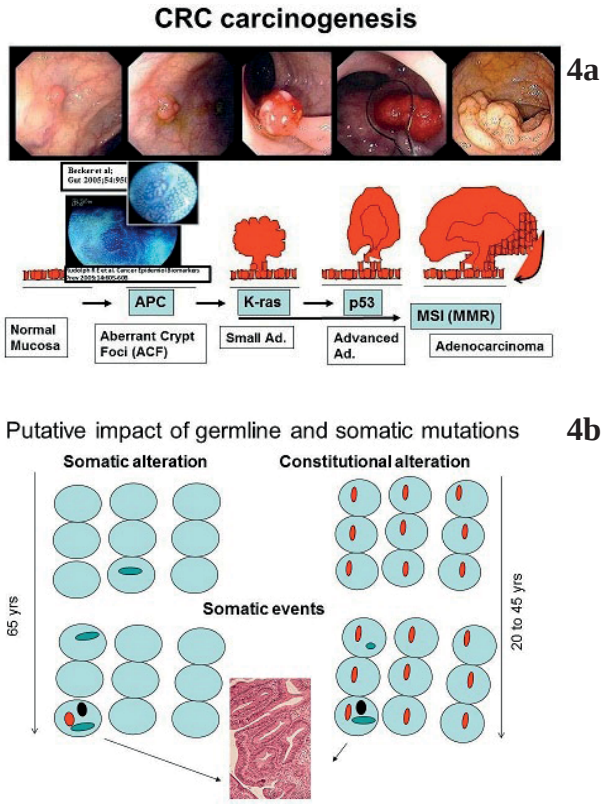

Fig. 4: 4a: Colorectal carcinogenesis from normal to invasive cancer. Up: colonoscopy slides after cleaning preparation. Down: process of the accumulation of gene mutations is shown; high magnification showing aberrant crypt foci (ACF) needed to detect precancerous lesions in macroscopic normal mucosa.
4b. Putative role of constitutional germline and somatic DNA mutations in the occurrence of CRC.
In the case of constitutional germline mutation (right), the length of colorectal cancer (CRC) occurrence from the birth until the accumulation of DNA mutations is shortened compared to sporadic cases (left).
Figure 4a: Please change: "Carcinogenesis" to "carcinogenesis"; "Mucosa" to "mucosa"; "Crypt Foci" to "crypt foci"; "Ad." to "ad." And define "Ad". "Adenocarcinoma" to "adenocarcinoma".
Figure 4b: Please change to English from French.

Focal and diffuse alteration in the intestinal mucosa

Stool versus adherent bacteria composition may impact diffuse and focal injuries, respectively. In the mid-1970s and early 80s, Deschner and colleagues identified focal histologic lesions in colonic mucosa of experimental animals that were the earliest morphologic alterations which preceeded tumor development(32). Later they showed an association between this phenomenon and diffuse hyperproliferation in intestinal mucosa(33). The pitfalls in the use of colonic mucosal cell proliferation in assessing the potential risk for development into colonic neoplasia(34) and a neoplastic paradigm (e.g., ACF) based on focal areas along the colon have been reviewed(35). Consequently, quantitative changes in cell proliferation associated with a loss of the epithelium's ability to inhibit altered (gene mutated) progenitor cells into differentiation, have been shown to increase the risk of neoplastic development(36,37). In this schema (Figure 4), human microbiota plays an important role, of which microbiota have undergone tremendous research using animal models. The transplant of human microbiota into the intestines of conventional animals has been shown to

stimulate intestinal cell proliferation compared to germ-free animals. The latter observation as well as large antibiotic therapy accompanied with weight loss and mucosal atrophy have been discussed by Reikvan et al. (38). It is likely that this effect is direct, necessitating bacterial attachment to epithelial cells. However, an indirect long term effect through cytokine production producing a stimulatory effect on the epithelial cells is not excluded.

Host recognition of microbial species

Humans, mice, and other eukaryotes are equipped with an elegant repertoire of receptors, with each receptor recognizing specific conserved microbial patterns, such as components of bacterial cell walls or nucleic acids. These microbial sensors include several RNA helicases, lectin receptors, and Toll-like receptors (TLR)(39). These receptors are also expressed on intestinal epithelial cells, including endocrine cells, and on various mucosal immune cells. They initiate signaling cascades that involve mitogen-activated protein kinases (MAPK), the nuclear factor of kappa B (NF-κB), and interferon regulatory factors that in turn modulate apoptosis, proliferation, and cell migration either directly or via cytokines and/or hormones through a paracrine pathway. These mechanisms are in agreement with several clinical trials that have shown the importance of hyperproliferation in the normal mucosa of individuals at high risk for CRCs(40,41). The substantial role of hyperproliferation induced by microbiota as driving CRC has been best demonstrated in gene mutated animals. For instance, the ApcMin/+ mouse has multiple intestinal neoplasia alleles of the adenomatous polyposis coli gene. This mouse is a model for human familial adenomatous polyposis and has been shown to develop tens to hundreds of intestinal adenomas when raised in conventional or specific pathogen-free (SPF) conditions. However, mice raised in germ-free conditions exhibit approximately a 50% reduction in intestinal tumors(42).The susceptibility of animals to developing cancer also depends on whether bacteria can induce mucosal inflammation. More recently, a microbial composition has been found to influence the development of colitis-associated CRC. Similarly, in the newly developed azoxymethane (AOM)/IL-10−/− model in which intestinal inflammation occurs spontaneously from the lack of immunosuppressive IL-10, tumorigenesis has been initiated by using the colon-specific carcinogen AOM; these mice developed extensive intestinal inflammation and colonic adenomas under conventional or SPF conditions(43). However, in association with Bacteroides vulgates, these mice were shown to exhibit fewer tumors; those raised under germ-free conditions were devoid of intestinal inflammation and tumors.

Bacteria-induced DNA alteration

Whether some bacteria can induce DNA damage has not been documented. It has been suggested that enteropathogenic *E. coli possesses* the ability to down regulate DNA mismatch repair proteins and, therefore, could promote colonic tumorigenesis by directly favoring DNA damage(44). *E. faecalis* induces aneuploidy in colonic epithelial cells and aggressive colitis in mono-associated IL10−/− mice. Inhibitors of reactive oxygen and nitrogen species (RONS) prevent *E.faecalis*-induced aneuploidy (43). Thus the unique ability of this bacterium to induce RONS can lead to chromosomal instability in a susceptible host. Consequently, one needs to distinguish between indigenous intestinal bacteria that have the capability to drive epithelial DNA damage, those that contribute to the initiation of CRC and intestinal niche alterations that favor the proliferation or inhibition of tumor growth. In contrast to driver mutations in the genomes of cancerous cells, bacterial drivers may disappear from cancerous tissue as they are outcompeted by passenger bacteria with a growth advantage in the tumor microenvironment. Species such as *Bacteroides*, *Shigella*, *Citrobacter*, *E.coli* and *Salmonella* species may be considered bacteria drivers since they are more abundant in the early stages of CRC and adenomas. These bacteria disappear from cancerous tissue as the disease progresses. *Fusobacterium* species, *Streptococcus gallolyticus* subsp. *gallolyticus*, *Clostridium septicum*, *Coriobacteriaceae slackia* and *Collinsella* species, the genus *Roseburia* and the genus *Faecalibacterium* may be considered as passenger bacteria(13).

An alternative pathway by which microbiota may drive carcinogenesis effects would be through hormone and metabolic changes(45).

Indirect bacteria effects, energy balance and metabolism

A standard low-fat (SLF) diet changing to a high-fat diet (HFD) within weeks has been shown to be associated with a shift in the balance of the two dominant phyla, *Bacteroidetes* and *Firmicutes* due to the expansion in one group of *Firmicutes*, the *Mollicutes*. This trait can be transferred by the transplant of microbiota into

lean recipients. Similar changes in the proportion of *Firmicutes* and *Bacteroidetes* have been identified in overweight and obese humans, in genetically obese mice, and in obesity-resistant mice fed a HFD diet (45,46). These highlight the critical role of specific members of the commensal microbiota in the development of metabolic- and metabolic-associated CRC. For instance, hyperleptinemia or hyperinsulinemia have been shown to increase the number of preneoplastic lesions in Apc-/-mice treated with exogenous leptin or in db/db rat treated with AOM (47,48). In the first model, APC gene alteration is constitutional, and occurs in all cells within crypts including stem cells; these cells are submitted to pharmacologically induced hyperleptinemia. In the second model, AOM induces somatic gene alteration (i.e.beta cathenin or CTNNB1) including stem cells; these cells are submitted to an endogenous hyperleptinemia that results from alteration of leptin receptor. In both models, leptin should be considered as a stimulatory factor of epithelial cell proliferation. Serum leptin levels are elevated in obese individuals. Obese males of all ages and postmenopausal women are at increased risk for CRC(49). Visceral associated fat (VAF), a stigma of various hormonal disorders, is considered a source of presumptive metabolic risk factors for colon cancer in mice. These develop hyperinsulinemia and preneoplastic colonic mucosal changes more often than mice without visceral abdominal obesity. Insulin-like growth factors are mitogens and regulate energy-dependent growth processes, cell proliferation and inhibit apoptosis(50). Importance of association between insulin resistance and colonic adenomas and cancers has been highlighted (Sobhani et al., 2010; (49). These results are consistent with those from prospective and interventional studies that have been performed in obese individuals, which have shown higher colonic cell proliferation and elevated number of ACFs in the colonic mucosa(50,51).

Bacterial enzyme activity

The production of bioactive carcinogenic compounds from environmental factors (diet, chemical agents) may be obtained through enzyme activities (β-glucuronidase, β-glucosidase, azoreductase, and nitroreductase). AOM is first hydrolyzed in the liver to methylazoxymethanol and conjugated with glucuronic acid before it is transported to the intestine through bile secretion of glucuronic acid-conjugated methylazoxymethanol. Similarly, bacterial β-glucuronidase spontaneously yields the highly reactive methyl carbonium ion, a carcinogenic form from AOM, while inhibition of β-glucuronidase significantly reduces the ability of AOM to induce tumors in rats(52).

Chronic inflammation

Numerous cancers arise from sites of infection, chronic irritation, and inflammation. The strongest association of chronic inflammation with malignant diseases is found in IBD of the colon(53) with a lifetime incidence of 10% (54). Thus, those patients who suffer from chronic IBD (Crohn's disease, ulcerative colitis) are considered at high risk of acquiring CRC. Such individuals should undergo colonoscopies every two to three years even in case of clinical remission of the IBD. Special zoom endoscopy and/or coloration process should be used to enhance the sensitivity of the colonoscopy for detecting precancerous lesions such as ACF and/or high grade dysplasia. Although the microbiota influences the development of colitis-associated CRC, the extent of host microbial recognition in this process is still unclear. A recent study in animals has clearly shown that colitis can promote tumorigenesis by altering microbial composition and inducing the expansion of microorganisms with genotoxic capabilities(52). This involves two different groups of bacteria, those which induce chronic inflammation and those favoring carcinogenesis. Monocolonization with the commensal *Escherichia coli* (*E. coli*) NC101 has been shown to promote invasive carcinoma in azoxymethane (AOM)-treated Il10(-/-) mice. Deletion of the polyketide synthase (pks) genotoxic island from *E. coli* NC101 decreases tumor multiplicity and invasion in AOM/Il10(-/-) mice without altering intestinal inflammation. The role of bacteria stimulating proinflammatory mediators is therefore important. Wu and colleagues(55) have shown that the intestinal flora may promote colon tumor formation in gene mutated mice model via immunologic mechanisms. They have demonstrated that *Bacteroides* enterotoxigenic fragilis, a common human commensal bacterium, induces cancer by a TH17-dependent pathway mechanism. These researchers have observed more ACFs and cancers in animals treated with this bacterium compared to the controls; this effect could be suppressed by an anti-IL17 antibody. It is likely that the relationship between activation regulatory T cells, overexpression TH17 cells in the colonic mucosa and overexpression of the adherent Bacteroides group to mucosa impact the development of colon cancer. These observations

are consistent with inflammatory and immune cell infiltration in the homologous normal mucosa of cancer patients in our human colonoscopy series compared to normal mucosa in individuals with normal colonoscopy results(16). However, whether this dysbiosis directly causes colon cancer or is a result of confounding factors from the environment remains to be investigated. In an experimental study performed on mice (personal unpublished data) we have observed that colon cancer patients' fresh stools exert higher proliferative effect on the intestinal mucosa in germ-free animals in which ACFs are also enhanced compared to the control germ-free mice after the transfer of gender- and age-matched normal individuals' fresh stools. Quantitative PCR measured all bacteria and main groups of bacteria and did not reveal significant differences between recipient mice during the experimental period, which have suggested that these main groups of bacteria are not likely the cause of the observed intestinal mucosa changes. However, 16sRNA gene pyrosequencing results showed significant differences between colon cancer and normal colon individuals' stools at baseline. Thus, we have proposed the possible involvement of other bacteria groups or species (Sobhani et al, 2011c).

PERSPECTIVES IN CRC RISK CALCULATION

Mortality due to CRC and its associated global cost to health care systems justify deployment of screening programs based on biological testing prior to colonoscopy or direct access to colonoscopy in average-risk and higher-risk populations(56).

Age-related risk in the general population

About 3% to 6% of the population aged 50 to 75 years old present with either adenomatous polyps of varying sizes or cancer(57). Adenomatous is a precancerous phenomenon with generative process requiring interactions of various factors, mainly from the environment. In this regard, time to exposure is important since 10 to 15 years are likely needed for polyps to evolve into cancer if they are not removed. Of interest, colonoscopy as a tool for mass screening might be reconsidered regarding the age and mucosal polyp phenotypes(58).

Gene- and familial-related risks

Although no more than a dozen genes are involved in early colon carcinogenesis, less than 5% of CRC result from a constitutional mutation. In these cases, the relatives who carry the mutated gene remain at very high risk of developing cancer. The risk for this group to develop cancer varies from 25% up to 100% depending on the mutated gene, age and location of the tumor or prenoplastic lesions. Even though the hereditary germline mutation constitutes the main risk factor (Table 1), the actual development of cancer among the carriers in these family members is likely dependent on environmental factors. In addition, about 10% to 15% of individuals with CRC and/or adenomas have other affected family members whose conditions do not fulfil the criteria for a constitutional gene-related carcinoma. A simple family history of CRC that is defined as one or more close relatives with CRC in the absence of a known hereditary colon cancer confers a two- to six-fold increase in CRC risk. A personal history of adenomatous polyps confers a 15% to 20% risk of subsequently developing polyps and increases the risk of CRC in relatives with diagnosed adenomas before the age of 60. The estimated relative risk for this population is 2.59 (95% CI: 1.46–4.58) (59). Interestingly, the RR (relative risk) of CRCs for spouses also appears to be higher than the RR in the general population (57).

Putative roles of genetic and environment factors have been researched in two studies performed in twins and spouses. After analysis of the environment factors, the researchers have explained the degree to which hereditary factors contribute to familial CRCs(60,61) and support intra-familial transmission of cancer. Briefly, familial clusters account for approximately 20% of all CRC cases in developed countries, while highly penetrant Mendelian CRC diseases only contribute up to 5% of familial cases. Overall, shared environmental factors may contribute

Table 1: Risk of CRCs among gene mutation carriers.

Syndrome	Relative Risk	Age of onset
FAP	90%	45 yrs
Attenuated FAP	69%	80 yrs
Lynch	40% to 80%	75 yrs
MYH-associated polyposis	35% to 53%	50 yrs or more
Peutz-Jeghers	39%	70 yrs
Juvenile polyposis	17% to 68%	60 yrs

Although individuals with constitutional gremline mutation are presenting clearly with higher risk than the average risk in the general population, age might impact the risk depending on time to exposure to environmental factors.
Ref: Guidelines of National Cancer Institute at the National Institutes of Health http://www.cancer.gov/cancertopics/pdq/genetics/colorectal/HealthProfessional).

to 90% or more of the CRC cancers.

Environment-related risk

Although CRC remains one of the most common cancers its incidence rate varies up to 10-fold in both sexes worldwide, the highest estimated in Western Europe, Australia and New Zealand, with the lowest in Africa and India. Increased consumption of red meat and animal fat increases CRC, whereas greater consumption of fiber reduces this risk(62). The link between a Western diet and elevated risk of colon cancer has been established for quite some time(63). The effect of diet on colonic mucosa can either maintain colonic health or promote chronic inflammation(62). The first attractive explanation of this observation was that dietary constituents might influence colon cancer prevalence. It was hypothesised that these might be involved in CRC carcinogenesis through the microbial flora of the gut. Microbiota change and dysbiosis have been documented in colon cancer or adenomatous polyp patients compared to control groups(18,19,62,64,65).

Acquired immuno-compromised individuals

Numerous publications suggest that higher risks should also be considered for individuals who may be immunocompromised such as patients who suffer from AIDS or those who take long term immunosuppressive drugs due to kidney, liver, heart, and pancreas transplantations (66,67). Whether this risk is due to an alteration in the immune system or favored by over selection of specific bacteria requires further investigation.

Anti-cancer bacterial effect

Exclusion of opportunistic pathogens by commensal bacteria may represent a natural defense against gastrointestinal diseases, including CRC. Probiotic bacteria (*Lactobacillus* sp. and *Bifidobacterium* sp.) exert anticarcinogenic effects in part by inactivating microbial enzymes. For example, probiotic lactic acid bacteria including *L. casei* and *L. acidophilus* can decrease the activity of β-glucuronidase, azoreductase, and nitroreductase. *Bifidbacterium longum* reduces AOM-induced ACF, which correlates with a decrease in AOM-activating β-glucuronidase activity. *Lactobacillus* sp. and *Bifidobacterium* sp. can inhibit DNA damage and tumorigenesis induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and 1,2-dimethylhydrazine (DMH)(52). In general, microbial fermentation of dietary fibers leads to production of short chain fatty acids (SCFA) acetate, propionate, and butyrate. These are absorbed by colonocytes and used as primary sources of energy. They provide protection during the early stages of tumorigenesis(68).

The use of probiotics to prevent mild inflammation in the colonic mucosa via bacteria change would be a promising future therapeutic treatment.

SYNTHESES OF RECENT HYPOTHESES AND DATA

We propose that the composition of microbiota can shape a healthy immune response or predispose it to disease. Many factors can contribute to dysbiosis and include lifestyle, medical practices, and likely host genetic phenotype. Mutations involved in immune regulatory mechanisms or pro-inflammatory pathways could lead to unrestrained inflammation in the intestine. It is possible that inflammation alone influences the composition of the microbiota, skewing it in favor of pathobionts. Alternatively, a host could 'select' or exclude the colonization of particular organisms.

This selection can be either active as would be the case of an organism recognizing a particular receptor on the host or passive where the host environment is more conducive to fostering the growth of select organisms. In some conditions selection of pathobionts by the host could tip the balance in favor of inflammation. Diet, toxic components, and stress also have the potential to induce direct somatic gene alteration in the colonocytes or alternatively influence microbiota. The overall results would be the failure to inhibit epithelium renewal in a mucosa with stem cells in which crucial genes are altered.

Of note, the composition of microbiota should be fully analyzed by metagenomic sequencing on whole bacteria genes in order to identity microorganisms that promote health and/or disease. These studies are currently in progress in Europe and the US. Potential dysbiotic microbiota possibly found in CRC patients could be tested in experimental models. Does the shift in the microbiota directly alter the course of disease? This important question requires animal studies. Is the information yielded by the metagenomic approach sufficient to initiate functional experiments where a cause/effect relationship could be established using animal models? What would be the outputs of functional studies using dysbiotic microbiota that have been obtained from germ-free animals and various disease states such as inflammation and CRC? Although identification of microbial consortia associated with

particular pathological conditions represents an important milestone, this critical step is not sufficient to fully understand the role of these microbiota in health and disease. Thus, it is crucial to study the possibility and feasibility of manipulating human microbiota and its metabolic capacity as an innovative approach to treating and preventing CRC.

CONCLUSION

Genetic alterations and environmental factors are both involved in colon cancer genesis. Growing data are now available on the involvement of nutrients, hormones and metabolic disorders that favor colon cancer growth. Colon microbiota should be considered a novel window for analyzing exhaustive factors from the environment. Although there is no real evidence that bacteria might directly induce mutation in colonocytes, changes in energy uptake, metabolic disorders, and stimulation of epithelial cells and reduction of protective bacteria are possible ways by which carcinogenesis might be facilitated in humans.

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