The interplay between the microbiome and the adaptive immune response in cancer development

Ther Adv Gastroenterol
2016, Vol. 9(4) 594–605
DOI: 10.1177/1756283X16635082

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***Abstract*:** The data from different studies suggest a bacterial role in cancer genesis/progression, often modulating the local immune response. This is particularly so at the mucosal level where the bacterial presence is strong and the immune system is highly reactive. The epithelial surfaces of the body, such as the skin and mucosa, are colonized by a vast number of microorganisms, which represent the so-called normal microbiome. Normally the microbiome does not cause a proinflammatory response because the immune system has developed different strategies for the tolerance of commensal bacteria, but when these mechanisms are impaired or new pathogenic bacteria are introduced into this balanced system, the immune system reacts to the microbiome and can trigger tumor growth in the intestine. In this review, we discuss the potential role of the bacterial microbiome in carcinogenesis, focusing on the direct and indirect immune adaptive mechanisms, that the bacteria can modulate in different ways.

Keywords: adaptive immune response, bacteria, colorectal cancer, microbiome, Th17

Introduction

The involvement of infectious elements in the cancer etiology has recently polarized the attention of many researchers. In the past, in 1890, the Scottish pathologist William Russell [Russell, 1890] reported circumstantial evidence for the bacterial cause of cancer. Actually, the data from different studies have strengthened the theory proposed by Russell suggesting a bacteria role in carcinogenesis and cancer progression, often interfering with and modulating the local immune response [Shahanavaj *et al.* 2015]. This is especially true at the mucosal level, where the bacterial presence is strong and the immune system is highly reactive. Actually, the epithelial surfaces of the body, such as the skin and the mucosa, are colonized by a vast number of microorganisms, which represent the so-called normal microbiome (Figure 1).

The human microbiome is composed of organisms belonging to the domains of the Bacteria, Archaea, Eukarya and their viruses. Most of the commensal bacteria are symbiotic, however, after translocation through the mucosa or under specific conditions (e.g. immunodeficiency), commensal bacteria can cause pathology.

The intestinal mucosa is the largest surface of the body that is constantly exposed to dietary and bacterial antigens. The gastrointestinal tract of mammals harbors a bacterial community containing trillions of members, including thousands of different species. There are more than 50 bacterial phyla on Earth, but the human gut-associated microbiome is dominated by four main phyla: Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria [Eckburg *et al.* 2005; Mahowald *et al.* 2009] (Figure 1). A wide proportion, about 70%, of the human microbiome is composed of bacteria that cannot be cultivated by current microbiological methods. The traditional culture-based methods capture less than 30%, of our bacterial microbiome [Fraher *et al.* 2012]. However, today, genomic next-generation sequencing analysis has been essential in defining and understanding the bacterial microbiome and metagenome, and their key role in metabolism,

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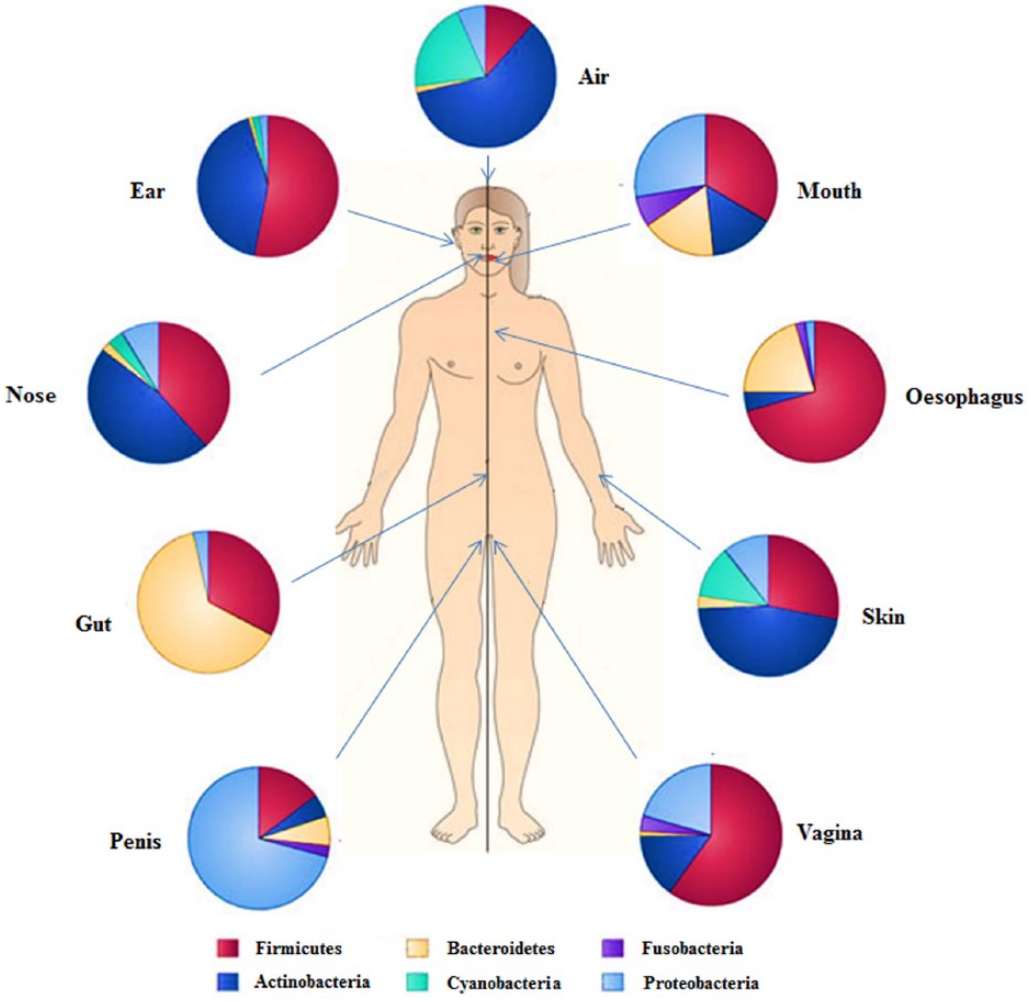

Figure 1. Different sorts of bacteria living in different places in and on the body.

inflammation and cancer progression [Kau *et al.* 2011; Human Microbiome Project Consortium, 2012].

The human microbiome is essential to body physiology, as it could produce an enormous quantity of molecules able to interact with the host. In particular, the bacteria of the gut microbiome represent a natural defense against colonization with pathogens and furthermore, they break down indigestible dietary components (e.g. vegetal polysaccharides) [Sonnenburg *et al.* 2004]. The bacteria communities are separated from the internal gut milieu by a single layer of epithelial cells, which not only is a physical and chemical barrier, but also balances the crosstalk between the external environment and immune host system. In addition, the epithelial surfaces have evolved protective mechanisms to counteract microorganism invasion. Effective innate and adaptive immune responses protect the mucosa and the internal environment of the human body. Almost 80% of the immunologically active cells belong to the mucosal-associated immune system. Most of these cells are present in the gastrointestinal tract, where immunogenic agents, such as food and components of the bacterial flora, are at the highest level with respect to other parts of the body.

Normally the microbiome does not cause a proinflammatory response because the immune system has developed various mechanisms to tolerate the commensal bacteria and preserve the homeostasis, but when these mechanisms are impaired or new pathogenic bacteria are introduced into this balanced system, the immune system reacts to the microbiome and can trigger tumor growth in the intestine [Schwabe and Jobin, 2013].

In this review we discuss the potential role of the bacterial microbiome in carcinogenesis, focusing on the direct and indirect immune adaptive mechanisms that the bacteria can modulate promoting, supporting or counteracting cancer development.

Microbiome's role in tumor development

Recent evidence suggests that human disease is attributable not only to a single bacteria but also to global changes in the host microbiome [Turnbaugh *et al.* 2006; Smith *et al.* 2013]. Different studies in germfree animals associated with bacteria have revealed evidence for tumor-promoting effects of the microbiome in spontaneous and genetically induced cancers in several organs, including skin, colon, liver, breast and lungs [Dapito *et al.* 2012; Sacksteder, 1976; Dove *et al.* 1997].There are also data showing the contrary view that gut microbiota have a central role in limiting chemically induced injury and proliferative responses that lead to tumor development in germ-free animals [Zhan *et al.* 2013]. In 1975, Reddy and colleagues first linked the gut microbiome to intestinal cancer development, establishing that only 20% of genetically modified germ-free rodents develop chemically induced colon cancer. In contrast, the tumor incidence in conventional rats with a normal microbiome was about 90% with several neoplasms [Reddy *et al.* 1975]. Vannucci and colleagues confirmed these data showing that germ-free rats, compared with genotypically similar animals with a normal microbiome, develop smaller tumors, as spontaneously as after chemically induced carcinogenesis [Vannucci *et al.* 2008]. Moreover, in colitis-associated cancer and adenomatous polyposis coli (APC)-related colorectal cancer, germ-free mice display decreased tumor formation and less oncogenic mutations [Rakoff-Nahoum and Medzhitov, 2008]. In addition, antibiotics depletion of the gut microbiota in mice reduces cancer development in the colon and the liver [Dapito *et al.* 2012; Yoshimoto *et al.* 2013; Chen *et al.* 2008; Klimesova *et al.* 2013] as does the eradication of specific pathogens in humans and in mice [Lee *et al.* 2008; Wong *et al.* 2004].

All these studies provide compelling evidence for the microbiome's role in tumor growth. Probably, the germ-free rats can develop a more active anticancer immune response in the absence of the physiological inflammation induced by the presence of a commensal microbiome. For this purpose, Sears and Pardoll [Sears and Pardoll, 2011] suggest that 'alpha bugs' (certain microbiome members possessing unique virulence traits), such as enterotoxigenic *Bacteroides fragilis* (ETBF), are directly pro-oncogenic and able to remold the mucosal immune response and colonic bacterial-removing species that protect against cancer thus promoting cancer growth.

The 'bacterial driver–passenger' model was first suggested by Tjalsma and colleagues [Tjalsma *et al.* 2012] to describe the microbial involvement in the development of colorectal cancer. According to this model, distinct indigenous intestinal bacteria, called 'driver bacteria' would create DNA damage and drive genome instability to initiate the first phases of cancer progression. As a consequence, the bacterial drivers (alpha bugs and their helpers) are gradually replaced by gut commensals with either tumor-promoting or tumor-suppressing properties (bacterial passengers). This model proposes that disease progression causes changes in the microenvironment as a result of the increasing tumor, resulting in a reformed selective pressure on the microbial community.

The importance of the human microbiome in host health and disease is also described by the 'keystone pathogen' hypothesis. The term 'keystone' has been introduced in the ecological literature to characterize species whose effects on their communities are disproportionately large relative to their abundance and which are thought to form the 'keystone' of the community's structure. This hypothesis supposes that some low-abundance microbial pathogens can orchestrate inflammatory disease by remodeling a normally benign microbiome into a dysbiotic one [Hajishengallis *et al* 2012].

Finally, inflammatory responses elicited by bacteria were demonstrated to be able to enhance cancer progression [Fukata and Abreu, 2008]. Some bacteria are able to induce alterations of mucosal permeability, facilitating the translocation of bacteria and bacterial toxins (e.g. lipopolysaccharide). Inflammation contributes to the development, progression, and treatment of cancer, but it remains unclear whether commensal bacteria affect inflammation in the sterile tumor microenvironment. An increasing number of studies demonstrated the role of inflammation in establishing conditions that can deeply alter local immune

responses and thus, tissue homeostasis. In particular, it is well documented that inflammatory mediators [such as interleukin (IL)-1, tumor necrosis factor-α (TNF-α), IL-8, nitric oxide or prostaglandin-2 derivatives] and molecules of the inflammatory pathways, are involved in a progressive interplay between the immune cells and tissue cells undergoing transformation [Mantovani *et al.* 2008].

The role of adaptive immune response in cancer progression

Vertebrates have evolved the machinery of adaptive immunity to counteract the pathogens that evade or overcome the innate immune defense. Normally the components of the adaptive immune system are silent; however, when activated, these components 'adapt' to the presence of infectious agents by activating, proliferating, and creating potent mechanisms to neutralize or eliminate the microbes. However, some of these responses can be protumorigenic.

There are two types of adaptive immune responses: humoral immunity, mediated by antibodies produced by B cells, and cell-mediated immunity, mediated by T cells, that are divided into two subsets: the $CD8^+$ (CTL) and the $CD4^+$ or T helper (Th). An effective antitumor immune response requires the involvement of both $CD4^+$ and $CD8^+$ T cells [Schreiber *et al.* 2011]. The role of $CD4^+$ T cells in antitumor immunity has recently been extensively studied in preclinical animal models and in clinical cancer patients. $CD4^+$ T cells are critical for priming tumor-specific $CD8^+$ T cells and for the secondary expansion and memory of $CD8^+$ T cells [Janssen *et al.* 2003]. However, the discovery of regulatory T cells (Treg) and Th17 cells not only updated the classical Th1/Th2 paradigm of Th cell differentiation, but also markedly revolutionized the concept regarding the role of $CD4^+$ T cells in antitumor immunity [Wang *et al.* 2012]. It has been demonstrated that tumor-infiltrating Tregs induce an immunosuppressive microenvironment, preventing an effective antitumor immunity and becomes a major obstacle to the success of anticancer immunotherapy [Curiel, 2008]. Furthermore, the functional contribution of human Th17 cells to tumor immunity remains controversial since both protumor and antitumor effects have been observed varied among tumor types [Wilke *et al.* 2011].

In addition to protection against cancer cells and pathogens, acquired immunity is essential for the establishment of complex bacterial communities in the gut.

Mechanisms of microbiome-driven carcinogenesis substantially differ between organs and the microbiome can modulate tumor development through direct and indirect actions. The microbial products can directly promote tumor growth or indirectly, that is the bacteria *per se* are not able to promote tumor progression unless they interact with the immune system. Finally, it is also possible that deficiencies in specific mechanisms of the immune response allow the expansion of certain bacteria, which activate a protumorigenic immune response.

The following paragraphs describe how the microbiota influence the adaptive immune response and, in turn, promote or counteract tumor development.

Direct responsibility of bacterial factors in cancerogenesis

Gastric cancer represents the most studied example of carcinogenesis closely related to infection by a specific bacterial pathogen, *Helicobacter pylori* [Lofgren *et al.* 2011; Peek and Blaser, 2002; Fox and Wang, 2007]. *H. pylori*, identified as a carcinogenic pathogen [Parkin *et al.* 2002], colonizes the human stomach and duodenum, leading to the sequential development of gastritis, gastric ulcer, atrophy and finally gastric cancer [Fox and Wang, 2007; Amedei *et al.* 2014]. *H. pylori* infection contributes to global cancer mortality with gastric cancer occurring in 1–3% of infected individuals [Fox and Wang, 2007]. *H. pylori* producing gastric atrophy and hypochlorhydria, renders the stomach susceptible to bacterial overgrowth, and subsequently increased bacterial conversion of dietary nitrates into carcinogens [Lofgren *et al.* 2011].

In addition, *H. pylori* infection is associated with the lymphoid hyperplasia of gastric mucosa that represents a preneoplastic condition of the mucosa-associated lymphoid tissue (MALT), which may evolve to a B-cell lymphoma [Munari *et al* 2011], where the anti-*H. pylori* immune response, seems to have a key role, in particular the macrophages and the Th lymphocytes [Amedei *et al.* 2014]. Recently, Munari and colleagues showed how *H. pylori* infection induces

a Th17 response [Munari *et al* 2014]. They studied the expression of cytokines in infected patients with chronic gastritis focusing on a role for B-cell activating factor (BAFF; CD257) in promoting Th17 differentiation. High levels of IL-17 and BAFF were present in gastric mucosa of *H. pylori*-positive patients with chronic gastritis and BAFF was especially abundant in macrophages and monocytes. Exposing monocytes to BAFF triggered reactive oxygen species (ROS) accumulation and also resulted in the production of IL-6, IL-1b, IL-23 and TGF-β. Monocytes exposed to *H. pylori* produced pro-Th17 cytokines and contribute directly to drive the gastric milieu in chronic gastritis. *H. pylori* affects the tumor microenvironment by increasing the IL-17A secretion and high levels of serum IL-23A in gastric cancer patients correlated with poor prognosis.

Recently, we have investigated the effector functions of gastric tumor-infiltrating lymphocytes specific for the *H. pylori*-secreted peptidyl prolyl cis, trans-isomerase (HP0175) [Amedei *et al.* 2014]. Tumor-infiltrating lymphocytes from patients with gastric adenocarcinoma, stimulated *in vitro* with HP0175, produced high levels of IL-17 and IL-21. T-cell clones derived from these populations were then tested for cytolytic ability and their ability to provide help to monocytes. While the cytolytic ability was poor, all of the Hp0175-specific Th17 clones were found to induce matrix metalloproteinase (MMP)-2 and MMP-9 production by monocytes. We hypothesized that HP0175-specific Th17 cells in the tumor drive the production of MMPs that promote angiogenesis and inflammation through IL-21 production.

Another example of tumors caused by specific bacterial pathogens is the gallbladder cancer associated with chronic *Salmonella enterica subsp.*, *Enterica serovar Typhi* and *Enterica serovar Paratyphi* infections [Caygill *et al.* 1994; Welton *et al.* 1979].

Both Gallbladder cancer and gastric MALT are examples of tumors triggered by adaptive immune responses against specific pathogens [Caygill *et al.* 1994; Wotherspoon *et al* 1993]. In addition, some lymphomas are associated with bacterial infections, such as *Campylobacter jejuni*, *Borrelia burgdorferi* and *Chlamydia psittaci*, and after antibiotic treatment a regression has been observed [Lecuit and Lortholary, 2004; Ferreri *et al.* 2012].

Fusobacterium nucleatum has recently been shown to directly promote intestinal tumorigenesis when its adhensin, FadA, binds to E-cadherin on epithelial cells and activates b-catenin signaling to promote epithelial cell proliferation [Rubinstein *et al.* 2013; Kostic *et al.* 2013]. Additional bacterial pathogens such as *Enterococcus faecalis*, enterotoxigenic *Bacteroides fragilis* and *Helicobacter hepaticus* promote cancer in animal models, but there is no clear epidemiological link to human carcinogenesis.

Finally, the microbiome has the capacity to directly modulate tumorigenesis through the production of specific toxins, such as genotoxins, that induce DNA damage responses, or tumor-promoting metabolites. For example, different bacterial toxins such as cytolethal distending toxin (CDT), cytotoxic necrotizing factor 1, *B. fragilis* toxin and colibactin, affect crucial cellular processes, implicated in tumorigenesis, and especially the responses to DNA damage [Arthur *et al.* 2012; Wu *et al.* 2009]. Microorganisms relevant to colorectal, gastric and gallbladder cancer (e.g. *E. coli*, *Helicobacter spp.* and *S. Typhi*) are CDT producers [Smith *et al.* 2013].

Various bacterial-derived metabolites, for instance hydrogen sulphide and superoxide radicals, may cause genomic instability [Carbonero *et al.* 2012; Huycke and Gaskins, 2004]. *Enterococcus faecalis* can generate large amounts of extracellular superoxide, which causes double-strand DNA breaks and chromosome instability leading to the development of colorectal cancer in mice [Wang and Huycke, 2007; Wang *et al.* 2012]

Antibacteria-specific immune response and tumor starting

Host-derived immune and inflammatory responses are an important driving force that shape the microbial community composition and, when altered, may contribute to dysbiosis. Some members of the microbiota alter the adaptive immune response and, in turn, promote cancer growth. One of the main instruments by which the microbiome can indirectly promote tumor growth are the Th17 cells. Commensal microbiota actively shape intestinal T-cell responses to promote homeostasis. For example, Th17 cells control microbial invasion in the gut, but specific compensatory mechanisms are required to regulate in turn the Th17 cells.

In the mammalian intestine the commensal bacteria induce IL-1β production to maintain a basal level of Th17 cells in the lamina propria under physiological conditions [Shaw *et al* 2012]. However, in response to pathogenic extracellular bacterial or fungal infections, large numbers of naive Th cells differentiate into Th17 under the influence of IL-1β, IL-6, IL-23 or TGFβ in mucosal surfaces of the gut and respiratory tract [Ouyang *et al.* 2008]. If those mechanisms are impaired, Th17 cells become pathogenic and can induce autoimmune disease and chronic inflammation. When stimulated with IL-6 and TGF-β, the antigen-activated $CD4^+$ T cells upregulate the transcription factor, retinoic acid receptor-related orphan receptor gamma t (RORγt) and secrete Th17-specific cytokines such as IL-17 and IL-22 [Korn *et al.* 2009]. Usually, the $CD4^+$ T cells that express RORγt increase tight junction formation and stimulate the secretion of microbicidal protein, contributing to the barrier function of the intestinal epithelium but they can have also a protumorigenic role [Korn *et al.* 2009].

As stated previously, compelling evidence suggests that Th17 cells and their cytokines have a strong tumorigenic potential, but the functional role of Th17 in cancer is ambiguous, it appears to have protumorigenic and antitumorigenic activities and the responses seem to depend on the cancer type [Grivennikov *et al.* 2012; Muranski *et al.* 2008; Murugaiyan and Saha, 2009]. In addition to IL-17A production, Th17 cells can secrete IL-17F, IL-21, IL-22, interferon (IFN)-γ and granulocyte-macrophage colony-stimulating factor (GM-CSF) [Zheng *et al.* 2008; Mitsdoerffer *et al.* 2010].

Th17 responses and mainly the IL-17 action itself, were initially thought to be proangiogenic, proinvasive and cancer growth promoting [Numasaki *et al.* 2003]. In a mouse model, the Th17 cells are able to promote colorectal cancer development, induced by colon inflammation [Wu *et al.* 2009]. Experiments with genetically predisposed mice (APCmin/+) crossed with IL-17A-deficient mice point out drastic impairment in intestinal tumorigenesis [Chae *et al.* 2010]. In addition, it has been shown that APCmin/+ mice that cannot respond to IL-17 develop fewer tumors in the colon [Grivennikov *et al.* 2012].

In humans, the role of Th17 cells has been investigated in patients with different cancer types, including ovarian cancer, prostate cancer, and many others [Yang *et al.* 2009; Horlock *et al.* 2009; Zhang *et al.* 2008a, 2009; Wang *et al.* 2008; Dhodapkar *et al.* 2008; Charles *et al.* 2009; Derhovanessian *et al.* 2009; Inozume *et al.* 2009; Koyama *et al.* 2008]. Most of these studies have examined Th17 cells in peripheral blood, but Th17 cells may be induced in or recruited to the cancer microenvironment [Kryczek *et al.* 2007, 2009].

Above, we have affirmed that the intestinal microbiota promote colorectal cancer development. More direct proof for a role of bacterially stimulated tumor growth via Th17 cells comes from studies of enterotoxigenic *Bacteroides fragilis*. This human colonic bacterium secretes *B. fragilis* toxin (BFT) that causes human inflammatory diarrhea. Several mouse models, predisposed to develop gut tumors, indicate that between colonization of *B. fragilis* and nontoxigenic *B. fragilis*, only the first triggers colitis and induces colonic tumors [Wu *et al.* 2009]. In particular, *B. fragilis* induces selective colonic signal transducer and transcription-3 (STAT3) activation with colitis characterized by a selective Th17 response. Antibody-mediated blockade of IL-17, as well as the IL-23 receptor, inhibits *B. fragilis*-induced colitis, colonic hyperplasia and tumor formation. These results show a STAT3- and Th17-dependent pathway of inflammation-induced cancer by a common human commensal bacterium, providing a new mechanistic insight into human colon carcinogenesis. In other words, these data support the role of Th17 cells and their cytokines (IL-17 and IL-22) in intestinal carcinogenesis.

Further experimental conditions have confirmed the ability of the microbiome to modulate the Th17 response. The Th17 response upon contact with specific bacteria, stimulates the expansion of neutrophil cells, required for the clearance of invading bacteria [Aujla *et al.* 2007; Blaschitz and Raffatellu, 2010]. The Th17 response is important for protection against mucosal pathogens like *Klebsiella pneumonia* and *Salmonella typhimurium*. Mice deficient in Th17 cytokines show a serious pathology during infection with *Salmonella* or *C. rodentium*, with increased translocation of bacteria into lymph nodes. [Raffatellu *et al.* 2008].

Other bacteria species capable of activating Th17 are the segmented filamentous bacteria (SFB), belonging to nonculturable *Clostridia*-related

species, and flagellin-positive bacteria. These bacteria interact with the epithelial cells in the host epithelial barrier promoting chronic inflammation, mediated by IL-17 and IL-22 release, which likely favors the development of intestinal cancer.

Recently, SFB has been shown to support the generation of Th17 cells [Suzuki *et al.* 2004] and only in the animals colonized with SFB, were found strong Th17 responses [Gaboriau-Routhiau *et al.* 2009; Korn *et al.* 2009; Reigstad and Backhed, 2010; Ivanov *et al.* 2009]. Moreover SFB-induced Th17 cells exacerbate autoimmunity, such as arthritis, that could be tempered by treating mice with certain antibiotics that reduced Th17 cells [Kosiewicz *et al.* 2011].

Based on such interesting data, some investigators became motivated to target the microbiome in order to enhance Th17 and $CD8^+$ T-cell responses against cancer cells [Gajewski *et al.* 2013].

As reported previously, Th17 cells produce other cytokines such as IL-22, which has been linked to intestinal tumorigenesis in murine models [Kirchberger *et al.* 2013; Huber *et al.* 2012]. IL-22BP (IL-22 R) is regulated by the inflammasome and modulates tumorigenesis in the intestine and in human colon cancer [Jiang *et al.* 2013]. IL-22 is related to the development of human colorectal cancer by STAT3 activation. A procarcinogenic rule for IL-22 via the STAT3 pathway, was previously shown in several extracolonic cancers such as hepatocellular carcinoma and non-small cell lung cancer [Zhang *et al.* 2008b; Jiang *et al.* 2011]. Little is known about the IL-22 role in colorectal cancer, although polymorphisms in IL-22 were shown to be associated with an increased risk for cancer development [Thompson *et al.* 2010]. Furthermore, IL-22 in conjunction with IFN-γ can induce inducible nitric oxide synthase (iNOS) production and procarcinogenic nitric oxygen species in human colon carcinoma cell lines [Ziesché *et al.* 2007]

Finally, the cytokine IL-23 is produced by myeloid cells in response to different microbiome molecules, such as flagellin [Kinnebrew *et al.* 2012]. IL-23 has the ability to promote Th17-type responses characterized by the induction of the cytokines IL-17 and IL-22 [Liang *et al.* 2006]. IL-23 was found to be increased in human colon adenocarcinoma and, through induction of proinflammatory responses, promotes tumor growth [Langowski *et al.* 2006].

Antitumorigenic impact of microbiome

Although most of the studies show tumor-promoting activity of the bacterial commensal flora, antitumor effects have also been reported. In the past, antitumor effects were observed in patients with sarcomas, after the injection of heat killed bacteria or bacterial infections (termed Coley's toxin) [Starnes, 1992]. Subsequent studies assign these antitumor effects to specific bacterial components as NOD-like receptor (NLR) agonists and Toll-like receptor (TLR) agonists. The activation of innate immunity can convert cancer tolerance into an anticancer immune response [Garaude *et al.* 2012].

In addition, murine studies described an effective immune role in tumor surveillance and in the inhibition of proliferation and metastasis, which results in the cancer regression [Muranski *et al.* 2008; Kryczek *et al.* 2009; Martin-Orozco *et al.* 2009].

An anticancer microbiome-mediated effect was noted in hematopoietic tumors, through the Th17 response. In mouse models, the alkylating agent, cyclophosphamide, alters the microbiome composition, inducing dysbiosis in the small intestine and inducing the translocation of selected species of $Gram^+$ bacteria into secondary lymphoid organs. These bacteria stimulate the generation of a specific subset of 'pathogenic' Th17 cells and memory Th1 immune responses, which result in a potent cancer-suppressive Th17 response [Viaud *et al.* 2013].

In addition, in a recent study [Amedei *et al.* 2013], it was demonstrated that Th17 cells have a specific antitumor effector function in patients with pancreatic cancer, and that there are decreased levels of these cells in cancer tissue compared to healthy mucosa. These results suggest that the gut microbiome can help shape the anticancer immune response.

In other words, although some bacteria are able to induce Th17 cells, others could promote control of Th17 cells, limiting cancer development. *Lactobacillus*, *Bifidobacteria*, and *Clostridium* can induce $Foxp3^+$ Treg cells, a specific subset of regulatory T cells in the intestine [Honda and Littman, 2012]. These $Foxp3^+$ Treg cells,

through IL-10 secretion, can control the production of IL-17A and the proliferation of Th17 cells [Huber *et al.* 2011]. These interactions can have an anti-tumorigenic activity in the intestine. It is still not clear if this is due to the suppression of Th17 pro-tumorigenic activity, but Foxp3$^+$ Treg cells can actively block intestinal tumor growth through IL-10 secretion [Erdman *et al.* 2003; Erdman *et al.* 2005].

Also, the Bacteroides species have been shown to have immunomodulatory effects. In particular *Bacteroides fragilis* releases polysaccharide A (PSA), that seems to block intestinal inflammation. The colonization of mice with *B. fragilis* resulted in the suppression of a proinflammatory Th17 reaction and this modulation could be attributed to PSA because *B. fragilis* lacking PSA was unable to suppress Th17 response, and also failed to persistently colonize the intestine [Round *et al.* 2011].

Finally, a selected mixture of *Clostridia* strains, recently identified from the human microbiome, is found able to attenuate disease in preclinical models of colitis through the induction of Tregs [Atarashi *et al.* 2008], revealing a potential antitumorigenic role. These results suggest that therapeutic colonization with specific strains of human-associated bacteria may have the potential to reduce tumorigenesis.

In conclusion, it is plausible that the integration of such multiple signals through their cumulative effects on specific commensal microbial populations and the various immune arms will result in context-specific effects on cancer development, establishing the microbiome as a critical hub integrating host and environmental signals, that are implicated in tumor formation or suppression.

Conclusion

The growing awareness of the importance of the gut microbiome in health and diseases and recognition of the host–microbe mutualism at the immunological and metabolic levels become important for a better understanding of several pathologies, especially cancer, a plague of our century.

Multiple lines of evidence support the notion that the microbiome shapes the immune system and indirectly modulates the development of tumors through the immune system in different organs. A positive modulation of the composition and metabolic activity of the gut microbiota might represent an interesting approach to reduce the risk of carcinogenesis and cancer development.

Today, finding new methods to selectively manipulate the microbiome in order to stop tumor initiation and progression represents an exciting challenge. In the near future, high quality mechanistic experimental studies and interventional human studies might provide the scientific premise for the clinical use of probiotics for the therapy of cancer and other multifactorial human diseases. We need a better understanding of the mechanisms whereby altered immunity shapes microbiome composition and determines which microbes are present to embrace us with their metabolites.

Funding

This work was supported by grants from the regional contribution of "the Programma Attuativo Regionale (Toscana) funded by FAS (now FSC), the Italian Ministry of University and Research and the Foundation 'Ente Cassa di Risparmio di Firenze'.

Conflict of interest statement

The authors declare no conflict of interest in preparing this article.

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