Review

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Exploring the microbiota to better understand gastrointestinal cancers physiology

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Abstract: Gastrointestinal cancers account for around 40% of cancer-related deaths worldwide, representing a global health burden. There is a growing body of evidence highlighting the link between microbiota and gastrointestinal tumorigenesis and/or resistance to therapy. In the present manuscript, we reviewed the published studies on the relationship between the microbiota and the different gastrointestinal tumors, namely, gastric, colorectal and esophageal, including also the cancer of accessory organs such as liver and pancreas. There is an emergent interest in the manipulation of gastrointestinal microflora in order to understand the gastrointestinal tumorigenesis' processes and the establishment of chemoresistance mechanisms.

Keywords: accessory organs; chemoresistance; gastrointestinal cancers; metagenomics; microbiota.

Introduction

Gastrointestinal (GI) cancers represent a major global health burden as they account for the 25% of all the cancers and for 40% of cancer-related deaths worldwide [1]. Despite many advances in modern medicine, the lack of predictive biomarkers and the subsequent late diagnosis render the available therapeutic strategies, based mainly on surgery and conventional chemotherapy, poorly effective for patients with GI cancers.

Recently, the analysis of microbiota has attracted much attention, supported by the evidence that a specific profile of resident microbes contributes to both health and disease state in humans [2, 3]. Ninety-nine percent of 10^{14} microorganisms constituting the human microbiota with almost 2 kg in weight resides in the gut and includes at least 1000 different species of known bacteria with more than 3 million genes (150 times more than human genes) [4]; the remaining 1% of microorganisms are located in other organs and tissues such as genitals, skin and mouth. Only a small proportion (<30%) of our bacterial microbiota could be identified with culture-based methods, but the advent of new technologies using next-generation sequencing has filled this gap [4]. Most people share one third of the whole gut microbiota, whereas two thirds are specific for each individuals, also because its composition is rapidly and heavily modulated by the diet [5], by host genotype and by environment [6]. The gut microbiota is considered a "forgotten" or "hidden" organ, which is involved, through a molecular crosstalk with the host, in the maintenance of host energy homeostasis and in the stimulation of host immunity [7]. This fine regulation of homeostasis associated to the healthy status of the host is referred as eubiosis (from the Greek eu = good and bios = life), which occurs when the microbial species live in balance with the host contributing to maintain health. By contrast, a state of an unbalanced proportion of bacteria associated to an unhealthy status is called dysbiosis. The latter is more evident when the components of the microbiota are conveyed to different organs affecting their functionality. Indeed, bacterial structural components and bacterial metabolites impair the host's physiological processes. More recently, it has also become evident that microbiota is involved in the initiation and progression of cancer, and it modulates the response to cancer therapy and the susceptibility to toxic side effects [8]. In this review, we summarized the published studies taking into account the relationship between the microbiota and the different gastrointestinal tumors (Table 1).

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Table 1: Summary of the studies on the relationship between the microbiota and the different gastrointestinal tumors.

Type of cancer	Microorganisms associated	Biological matrix
Colorectal cancer	*Streptococcus gallolyticus* [9]	Feces
	Clostridium septicum [10, 11]	General infection
	Fusobacterium nucleatum [12–14]	General infection; cancer tissue
	Bacteroides fragilis [15]	Feces
	Escherichia coli [16–20]	Cancer tissue
	Enterococcus faecalis [21]	Feces
	Helicobacter pylori [22–29]	General infection
	Bacteroides fragilis, *Enterococcus*, *Escherichia-Shigella*, *Klebsiella*, *Streptococcus* and *Peptostreptococcus* increased, *Bacteroides vulgatus*, *Bacteroides uniformis*, *Roseburia* and *Lachnospiraceae* decreased [30]	Feces
	Clostridia decreased, *Fusobacterium* and *Porphyromonas* increased [31]	Feces
	Peptostreptococcus, *Porphyromonas*, *Mogibacterium*, *Anaerococcus*, *Slackia*, *Anaerotruncus*, *Collinsella*, *Desulfovibrio*, *Eubacterium* and *Paraprevotella* [32]	Gut lumen
	Bifidobacterium, *Faecalibacterium* and *Blautia* decreased, *Fusobacterium* increased [32]	Mucosal cancer tissue
Gastric cancer	*Helicobacter pylori* [33]	General infection
	TM7, *Porphyromonas*, *Neisseria* and *Streptococcus sinensis* decreased, *Lactobacillus colehominis* and *Lachnospiraceae* increased [34]	Gastric mucosa
	Klebsiella pneumoniae and *Acinetobacter baumannii* increased [35]	Gastric mucosa
	Lactobacillus, *Lachnospiraceae* uncultured, *Escherichia-Shigella*, *Nitrospirae* and *Burkholderia fungorum* [36]	Gastric mucosa
	Epsilonproteobacteria and *Helicobacteraceae* decreased, *Bacilli* and *Streptococcaceae* increased [37]	Gastric mucosa
Liver cancer	*Helicobacter hepaticus* [38]	Serum
	Escherichia coli [39]	Feces
Pancreatic cancer	*Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* [40]	Mouth wash sample
	Fusobacterium [41]	Pancreatic cancer tissue
	Neisseria elongata and *Streptococcus mitis* decreased, *Granulicatella adiacens* increased [42]	Saliva
	Corynebacterium and *Aggregatibacter* decreased, *Bacteroides* increased [43]	Mouth wash sample
	Helicobacter pylori [44–46]	General infection
Esophageal cancer	*Treponema denticola*, *Streptococcus mitis* and *Streptococcus anginosus* [47]	Esophageal cancer tissue
	Escherichia coli [48]	Esophageal cancer tissue
	Porphyromonas gingivalis [49, 50]	Oral wash samples; esophageal cancer tissue
	Tannerella forsythia, *Streptococcus pneumoniae* and *Neisseria* [49]	Oral wash samples
	Lautropia, *Bulleidia*, *Catonella*, *Corynebacterium*, *Moryella*, *Peptococcus* and *Cardiobacterium* decreased, and *Prevotella*, *Streptococcus* and *Porphyromonas* increased [51]	Saliva
	Clostridiales and *Erysipelotrichales* [52]	Gastric corpus tissue

Microbiota and colorectal cancer

Colorectal cancer (CRC) is ranked as the third most frequently diagnosed malignancy and the third cause of cancer-related mortality [53]. The disease typically results from the accumulation of multiple genetic mutations, which drive the progression from healthy epithelium to adenoma and to carcinoma [54, 55]. Despite the central role of genetics in the development of CRC, it is widely recognized that environmental factors such as diet and lifestyle strongly impact the pathogenesis [56, 57]. In particular, high consumption of red and/or processed meat, high-fat diet, low intake of fibers, heavy alcohol consumption, cigarette smoking and obesity represent well-known risk factors. Likewise, other risk factors are intestinal microenvironment conditions such as inflammatory bowel diseases and imbalances in gut microbiota [58].

The first hint of the involvement of intestinal microbiota in CRC was provided in 1975, with the observation that germ-free rats developed less tumors in response to chemical carcinogens compared to their conventional littermates [59, 60]. Studies in CRC patients have revealed a number of bacteria associated with the disease. The most known microorganism associated to CRC is *Streptococcus*

gallolyticus [9, 61–63], formerly known as *Streptococcus bovis*, whose infection (bacteremia or endocarditis) is found in up to 80% of patients [64, 65]. The proposed link between *S. gallolyticus* and colorectal carcinogenesis is through the increased expression of proinflammatory genes such as interleukin (IL)-1 and COX-2 and of the angiogenic chemokine IL-8 [66]. Similarly to *S. gallolyticus*, bacterial infection by *Clostridium septicum* has been clinically linked to CRC [10, 11], although the molecular bases of this link have to be elucidated. Another well-known microorganism associated with CRC is *Fusobacterium nucleatum*, which was found over-represented in colorectal tumor tissues [12–14]. One mechanism by which this bacterium would promote carcinogenesis is by activating E-cadherin/β-catenin signaling through binding with its FadA adhesin, thus increasing the expression of oncogenic and inflammatory genes [67]. Moreover, *F. nucleatum* would also impair antitumor T-cell-mediated immunity [68]. Activation of the E-cadherin/β-catenin signaling in the etiology of CRC, culminating in c-myc expression and proliferation [69], is also operated by the enterotoxin of *Bacteroides fragilis* [69, 70], whose gut colonization is increased in CRC patients with respect to healthy controls [15]. *Bacteroides fragilis* toxin was also reported to foster carcinogenesis by promoting inflammation [71]. Enhancement of proliferation and inflammation are also the main mechanisms underlying the linkage between *Escherichia coli* and CRC [16]. *Escherichia coli* is a commensal microorganism of the human gut, but some pathogenic strains (i.e. B2 and D phylogroups) that are adherent/invasive and produce toxins have been found to colonize the mucosal epithelium of CRC [16–20]. In more detail, *E. coli* phylogroup B2 produces cyclomodulins (such as colibactin), that are genotoxins able to produce DNA damage and/or to interfere with the cell cycle of the host cell [16, 17]. Colibactin was shown to promote colon cancer growth in an animal model by inducing cellular senescence and a senescence-associated secretory phenotype (SASP), which enhances proliferation [72]. Moreover, *E. coli* B2 infects tumor-infiltrating macrophages, resists killing and induces COX-2 expression and inflammation [19]. Induction of macrophage COX-2 expression was also reported as a consequence of reactive oxygen species (ROS) produced by *Enterococcus faecalis* [73], which was reported to be more abundant in the feces of CRC patients than in healthy controls [21]. ROS produced by *E. faecalis* damage colonic cell DNA and promote chromosomal instability, which may lead to CRC [21, 74]. The role of *Helicobacter pylori*, a leading cause of gastric cancer (GC), in CRC is still controversial [75, 76]. This infectious agent, whose habitat is the gastric mucus, has been associated to colorectal malignancy by several studies [22–29], despite a number of conflicting reports [77–79]. One likely explanation for this inconsistency may be in the different virulence of *H. pylori* strains [75]. A matter of debate is also the molecular mechanism by which *H. pylori* infection would favor the development of CRC [76]. One hypothesis is that *H. pylori* causes hypergastrinemia, and gastrin would have a mitogenic action on colonic cells [76]. Another proposed mechanism is the proinflammatory and pro-proliferative activity of the cytotoxin-associated gene A (CagA) of some *H. pylori* strains [28, 75, 76]. Both mechanisms confirmed by some studies, however, have been disproved by others [76].

In addition to the above-mentioned bacteria, other studies have reported different bacterial profiles between diseased and healthy people. Wang et al. [30] found *B. fragilis* and the genera *Enterococcus*, *Escherichia-Shigella*, *Klebsiella*, *Streptococcus* and *Peptostreptococcus* were enriched in feces of CRC patients compared to controls, whereas *Bacteroides vulgatus*, *Bacteroides uniformis*, *Roseburia* and butyrate-producing bacteria of the *Lachnospiraceae* family were more abundant in healthy controls. Ahn et al. [31] observed lower abundance of *Clostridia* and enrichment of *Fusobacterium* and *Porphyromonas* in stool samples from CRC patients with respect to disease-free subjects. A study by Chen et al. [32] examined the microbiota of both gut luminen and mucosal cancer tissue and found different profiles, *Peptostreptococcus*, *Porphyromonas*, *Mogibacterium*, *Anaerococcus*, *Slackia*, *Anaerotruncus*, *Collinsella*, *Desulfovibrio*, *Eubacterium* and *Paraprevotella,* were enriched in the lumen of patients compared to controls, whereas in cancer tissue, beneficial microbes such as *Bifidobacterium*, *Faecalibacterium* and *Blautia* were reduced, and *Fusobacterium* increased.

Microbiota and gastric cancer

Gastric cancer (GC) is ranked as fourth for incidence and second for lethality [80] among cancers. The development of the disease is a multifactorial process, in which both genetic and environmental factors, such as age, sex, diet, alcohol consumption and cigarette smoking may play a role [81, 82]. The main risk factor for GC, however, is chronic infection by *H. pylori* [33], a Gram-negative bacterium living in the gastric mucosa of half of human population [83]. Despite its wide diffusion in the population, only 1%–2% of *H. pylori* carriers develop GC [33, 84], likely because of the existence of different strains with different virulence, in addition to other individual susceptibility factors [33]. Several oncogenic mechanisms linking

H. pylori to GC development have been described, the most studied involves the CagA protein, encoded by the bacterial strains carrying the cagA pathogenicity islands. This protein, which is delivered into gastric epithelial cells, activates several pathways implicated in carcinogenesis [33, 85]: (i) promotion of proliferation signaling such as β-catenin, MAPK, PI3K-AKT and pathways [83, 86, 87]; (ii) interference with proapoptotic activities such as that of p53 and RUNX3 [86, 87]; and (iii) activation of the inflammatory NF-κB signaling [86, 87]. Another virulence factor, expressed by all *H. pylori* strains, is the VacA (vacuolating cytotoxin A) protein, which creates vacuoles in the host cells thus promoting apoptosis. Moreover, VacA is also reported to have immunosuppressive functions, which would enhance gastric tumor escape from the immune surveillance [33]. Since the discovery of *H. pylori* in 1983, awareness was acquired about the existence of a microbiota colonizing the stomach, formerly assumed to be sterile due to its acidic pH [84]. Indeed, five main bacterial phyla have been identified in the healthy human gastric microbiota, namely, *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Fusobacteria* [88]. According to some studies, *H. pylori* infection affects the composition of human gastric microbiota [34, 89], although conflicting papers report no significant difference between microbiota infected or non-infected with *H. pylori* [35, 36, 88]. Moreover, it is assumed that gastric bacteria other than *H. pylori* may also take part in the promotion of GC development, by producing reactive oxygen and nitrogen species and favoring inflammation [90]. Significant differences have been shown in the gastric bacterial profile of GC carriers versus non-cancer subjects [34, 36, 37]. A recent study comparing the gastric microbiota of GC and chronic gastritis patients showed a higher bacterial load and an increase of the microbial diversity in GC than in chronic gastritis [36]. This result was in line with the report of Eun et al. [37] who also observed an increase in microbial diversity but in contrast with other studies which reported a lower diversity in GC [34, 91]. Furthermore, despite no significant differences between GC and chronic gastritis at the phylum level, Wang et al. [36] found enriched in GC patients five bacterial genera, namely, *Lactobacillus*, *Lachnospiraceae* uncultured, *Escherichia-Shigella*, *Nitrospirae* and *Burkholderia fungorum*. In a previous study by Eun et al. [37], the class of *Epsilonproteobacteria* and the family of *Helicobacteraceae* were found decreased, whereas the *Bacilli* class, and the *Streptococcaceae* family were enriched in GC in respect to the gastritis and the metaplasia groups. Another study comparing the microbiota of non-atrophic gastritis, intestinal metaplasia and GC patients, revealed eight taxa differentially represented between the groups, with two species from *TM7* phylum, two *Porphyromonas* spp., one *Neisseria* sp. and *Streptococcus sinensis* showing a decreasing trend and *Lactobacillus colehominis* and *Lachnospiraceae* showing an increasing trend while progressing from gastritis to intestinal metaplasia to GC [34]. Khosravi et al. found two bacterial species, namely, *Klebsiella pneumoniae* and *Acinetobacter baumannii*, enriched in GC patients when compared with patients suffering from non-ulcer dyspepsia and peptic ulcer disease. This result, however, may be biased by the low number of GC patients analyzed compared to the other groups [35]. In disagreement with the aforementioned studies, Dicksved et al. [92] found no difference in the composition of microbiota between GC patients and controls, but this study was performed on a small number of subjects and did not took advantage of the current high-throughput sequence technologies [33, 84].

Microbiota and liver cancer

Primary liver cancer is the sixth most frequent neoplasia and a leading cause of death for cancer worldwide [93]. Hepatocellular carcinoma (HCC) is the dominant histology of liver cancer, representing about 80%–90% of all cases [14, 93]. It typically arises in the setting of chronic liver disease and cirrhosis, whose main risk factors are represented by chronic viral hepatitis B and C, heavy alcohol intake, ingestion of aflatoxins, diabetes, obesity and non-alcoholic fatty liver disease (NAFLD) [14, 94, 95].

The liver is anatomically and functionally connected to the gut, from which it receives approximately 70% of its blood supply through the portal vein. For this reason, it is constantly exposed to microorganisms, toxins, metabolites and other microbial products from the intestine [2, 96]. Several evidences support a role for the intestinal microbiota in the development of liver diseases, including HCC [97–99]. Alterations in gut microbiota have been described in obesity, NAFLD, alcoholic liver disease and in cirrhosis [98]. In cirrhotic patients, an increase in *Enterobacteriaceae*, *Streptococcaceae*, *Streptococcus* spp. and *Veillonella* and a decrease in *Bifidobacteria*, *Lachnospiraceae*, *Bacteroidetes* and *Firmicutes* were described [100–102].

Most reports linking microbiota with HCC come from experimental studies on animal models [14]; nevertheless, a number of clinical studies also exist concerning an association between some bacteria, mainly *Helicobacter* spp., and human liver cancer [14, 103–105]. *Helicobacter* spp. DNA was detected in liver samples from HCC patients [103–105] and in cirrhotic livers from HCV-infected

patients with or without HCC [106]. It is yet to elucidate whether *Helicobacter* has a causative role in the hepatocarcinogenic process [105, 107], although evidences from experimental models support this hypothesis [108, 109]. As for the species of *Helicobacter* associated with human HCC, some studies indicate *H. pylori* as the most common [103, 104]. Kruttgen et al. [109] investigated on whether *H. hepaticus*, which is strongly associated to HCC in murine models, would be also responsible for the human disease. They found no trace of *H. hepaticus* in stool samples from HCC patients, but their study was performed on a small number of patients with viral hepatitis-related HCC, and they could not rule out a role for this bacterial species in human HCC with different etiology [109]. On the contrary, in a report by Yang et al. [38], the infection by *H. hepaticus* in patients with primary HCC was demonstrated with both serological and molecular biological methods, suggesting that *H. hepaticus* may be involved in the pathogenesis of human HCC. Concerning the mechanisms through which *Helicobacter* spp. would influence HCC development, *H. hepaticus* is a producer of the cytolethal distending toxin, which has DNAse activity and would therefore impact on cell cycle [107, 110]; *H. pylori*, instead, produces the cytotoxins VacA and CagA, whose pathogenic functions have been previously described. Moreover, it is known that *Helicobacter* spp. are inducers of the proinflammatory NF-κB pathway [107], and inflammation plays a key role in hepatocarcinogenesis.

Another bacterium that has been related to HCC in human subjects is *E. coli*, which was found overgrown in the feces of cirrhotic patients with HCC compared to cirrhotic patients without cancer [39].

As a general mechanism, it has been demonstrated that gut microbiota concurs to hepatocarcinogenesis by means of soluble molecules named MAMPs (microbial-associated molecular patterns) and other bacterial metabolites, which reach the liver trough the bloodstream [99]. The main bacterial product responsible for the liver pathogenesis is lipopolysaccharide (LPS), a component of the Gram-negative bacterial cell wall, which binds to Toll-like receptor 4 (TLR4) expressed by hepatocytes, stellate cells and Kupffer cells resulting in the promotion of cell proliferation and inflammation [14, 99, 111]. Indeed, high levels of circulating LPS have been observed in patients with chronic liver diseases predisposing to HCC and antibody response to LPS was found significantly associated to the risk of developing HCC [112].

Moreover, a role for the bacterial metabolite deoxycholic acid (DCA) in the promotion of HCC development has been described in a mouse model of obesity-induced HCC. According to this model, DCA coming from the intestinal bacteria causes in hepatic stellate cells a SASP, that is to say the release of inflammatory and tumor-promoting factors that facilitate HCC development [113].

Microbiota and pancreatic cancer

With more than 330,000 deaths/year, pancreatic cancer (PC) is one of the deadliest cancers worldwide [1]. Among the GI cancers, PC is the one with the worst prognosis, with mortality approaching incidence [114] due to its biological aggressiveness and resistance to conventional therapies [115]. It is also defined as a silent killer because currently there is no screening biomarker that could predict the onset of the disease, the symptoms are unspecific and varied and the diagnosis occurs at advanced stage [116], thus affecting the efficacy of all the therapeutic strategies that are considered rather as palliative care. Less than 5% of PC patients is eligible for surgical resection, which increases the survival up to 5 years [1]. Risk factors for PC are obesity, alcohol, smoking, chronic pancreatitis, familiarity and type 2 diabetes [117, 118]. Recently, scientific papers demonstrated that periodontal disease, manifested by an inflamed oral activity due to pathogenic oral flora, are independent risk factors associated with the development of PC [119, 120]. More than 700 microbial species live within the oral cavity [121]. In a healthy oral flora, the predominant bacteria are *Streptococcus* and *Haemophilus* in the buccal mucosa, *Actinomyces* in the supragingival plaque and *Prevotella* in the adjacent subgingival region [121, 122], whereas *Porphyromonas gingivalis* belonging to the phylum *Bacteroidetes*, and *Aggregatibacter actinomycetemcomitans*, two species of bacteria linked to periodontal disease, are associated with a more than 50% increased risk of PC [40]. All these studies suggest that oral microbiota may play an important role not only in the periodontal disease and tooth loss but also in the etiology of PC, probably because after mastication oral bacteria enter the blood [123] and by providing MAMPs they can activate TLRs [124], which are involved in the innate immune response. Inflammation due to immunological response to oral bacteria and their toxins [125] has been shown to play a role in oral and GI carcinogenesis [126, 127].

Mitsuhashi et al. [41] reported that *Fusobacterium* species are independently associated with a worse prognosis and were detected in PC tissue with a different concentration between pancreatic tail, body and head [41], suggesting a role for *Fusobacterium* as a prognostic biomarker for PC patients. The shorter survival might be caused by the activation of inflammation processes due

to the increased production of ROS and inflammatory cytokines (e.g. IL-6 and Tumor necrosis factor) or through recruitment of tumor-infiltrating immune cells, generating a proinflammatory microenvironment as it has been seen for CRC [128]. However, when the oral microbiota was analyzed in salivary samples using bacterial 16S ribosomal RNA (16S rRNA) gene sequencing, higher levels of the phylum *Fusobacterium* and its genus *Leptotrichia* were found associated with a lower risk of PC [40].

Neisseria elongata and *Streptococcus mitis* were found, in oral flora, to achieve the highest discriminatory power between PC patients and healthy controls, whereas *Granulicatella adiacens* and *S. mitis* were significantly altered in patients with PC when compared with those with chronic pancreatitis and controls, with the levels of *G. adiacens* significantly elevated in PC patients relative to all non-cancer subjects [42]. The bacterial 16S rRNA gene sequencing performed on oral wash samples by Lin et al. [43] revealed that *Corynebacterium* and *Aggregatibacter* were less abundant in PC and pancreatitis groups when compared with controls, whereas *Bacteroides* were significantly more abundant in both PC patients and pancreatitis patients compared with control group. Scientific literature describes a role for *Bacteroides* spp. in the induction of inflammation at the intestinal level [129, 130] and our group found *Bacteroides acidifaciens* increased in a mouse model of xenografted PC, together with *Akkermansia muciniphila*, *Ruminococcus gnavus*, *Clostridium cocleatum* and *Escherichia* [131]. As *B. acidifaciens*, also *R. gnavus* is involved in inflammation as demonstrated by Png et al. [132].

Although inflammation is a beneficial response allowing pathogens elimination and the homeostasis of damaged tissues and organs, it is also well established that chronic inflammation plays a pivotal role in tumor development [133], in particular in PC which is typically an inflammation-driven cancer [134].

Lactobacillus is a commensal oral cavity bacterium that diminishes gingival inflammation and cariogenic periodontal pathogenic bacteria [135]. Thus, with the clearly established role for periodontal disease and associated periodontal pathogens in PC risk profiles, any measures to prevent periodontal pathogens may have a protective role to prevent PC.

Data from specific studies uncovered an association between the ubiquitous bacterium *H. pylori* and the risk of PC development [44–46], whereas some others reported no significant association [136]. Controversies still remain about a role for this microorganism in PC and about its putative pathogenetic mechanism [136]; nevertheless, it was provided *in vitro* evidence that *H. pylori* infection may increase malignant potential of human pancreatic cells by promoting the activities of proliferative and inflammatory factors such as AP-1 and NF-κB and increasing the secretion levels of the growth factor VEGF and the inflammatory chemokine IL-8 [137]. This suggest that *H. pylori* too may be involved in PC pathogenesis due to its ability to fuel inflammation.

Microbiota and esophageal cancer

Esophageal cancer ranks sixth among the deadliest cancers worldwide [1], owing its poor prognosis to late-stage diagnosis [138]. Two main histologies can be distinguished, namely, esophageal adenocarcinoma (EAC) arising from the glandular cells of the distal esophagus [139] and esophageal squamous cell carcinoma (ESCC) arising from the epithelial cells, with different geographical distribution [49]. Beside genetics, gastroesophageal reflux disease (GERD), alcohol and tobacco consumption, low fiber intake and obesity are known risk factors for this cancer [49, 140]. In particular, GERD likely predisposes to develop the Barrett's esophagus (BE), a condition of metaplasia representing a premalignant lesion often preceding the onset of EAC [141].

Recently, a contribution of the microbiota in the etiology of esophageal cancer has been suggested. The esophageal mucosa harbors its own microbiota, which is mainly composed by the genera *Streptococcus*, *Prevotella* and *Veillonella* in healthy humans [142, 143]. Alterations in the composition of esophageal microbiota have been described in BE, with an increase in Gram-negative bacteria (such as *Fusobacterium*, *Neisseria*, *Campylobacter*, *Bacteroides*, *Proteobacteria* and *Veillonella*) and a decrease in the Gram-positive *Streptococcus* [144, 145]. The Gram-negative microorganisms produce LPS which, by stimulating the TLR4, leads to the activation of the NF-κB signaling. Therefore, it is suggested that this change in microbiota composition establishes a condition of chronic inflammation predisposing to EAC [145].

The first study comparing the microbiota of normal and cancerous esophageal tissue by using culture-independent approach found a consistent colonization by the periodontopathic bacteria *Treponema denticola*, *S. mitis* and *Streptococcus anginosus*, of both tissues, leading the authors to speculate about a role for these microorganisms in the carcinogenic process [47]. This study, however, did not specify between EAC or ESCC. Given their different histology, indeed, different microbiota alterations have been associated to EAC and ESSC development. A paper by Blackett et al. [146], analyzed the

esophageal microbiota of patients with GERD, BE, EAC and the microbiota of controls, revealing an increased abundance of *Campylobacter* (mainly *C. concisus*) in GERD and BE in comparison with controls and EAC patients. Moreover, this study highlighted a strong association between *C. concisus* abundance and the expression of IL-18 [146], an IL stimulating the immune system that was reported to be associated to EAC [147]. Two years later, a study conducted on a rat model of EAC carcinogenesis revealed the presence of *E. coli* in 60% of BE and in 100% of EAC, but it was absent in tumor adjacent normal tissue, in dysplasia and in GERD. This finding was associated with an upregulation of TLRs 1–3, 6, 7 and 9 [48]. Surprisingly, a meta-analysis study performed by Islami and Kamangar [148] shows that the declining rate of *H. pylori* infection (a known risk factor for gastric, colon and PCs) coincides with a rising incidence of EAC in western countries, suggesting a protective role for *H. pylori* in EAC. Concerning the ESCC, Gao et al. [50] demonstrated the presence of *Porphyromonas gingivalis* in the esophageal mucosa of 61% of ESCC tissues, whereas it was undetected in normal mucosa. This result was replicated by a subsequent study, in which also other oral pathogens, i.e. *Tannerella forsythia*, *Streptococcus pneumoniae* and *Neisseria* were found associated to EAC [49]. The oral microbiota was found associated to ESCC risk also in a study conducted on a Chinese population, in which the bacterial profile of the saliva was traced in either ESCC, dysplasia patients and control subjects. A decreased microbial diversity in ESCC relative to the other groups emerged, together with a lower abundance of the bacterial genera *Lautropia*, *Bulleidia*, *Catonella*, *Corynebacterium*, *Moryella*, *Peptococcus* and *Cardiobacterium*. Conversely, *Prevotella*, *Streptococcus* and *Porphyromonas* resulted increased in ESCC compared to non ESCC subjects [51]. In a study by Yu et al. [91], the microbiota of the human upper digestive tract of patients with esophageal squamous dysplasia (ESD, a precursor lesion of ESCC) was compared to that of normal controls, revealing a lower microbial richness. A further study compared the gastric corpus microbiota of ESD and ESCC patients, showing an increased abundance of *Clostridiales* and *Erysipelotrichales* relative to controls, thus suggesting a role for gastric dysbiosis in the progression from ESD to ESCC [52].

Diet, microbiota and cancer therapy

It is well established that diet markedly influences the microbiota composition [149], and this is now widely considered an opportunity to modulate it in order to prevent or attenuate disease activity correlated to microbiota imbalance. Changes in diet can alter microbiota profiles within just 24 h, and in 48 h it is possible to reverse to the baseline once diet modifications are interrupted [150]. Western diet, for example, which is rich in animal proteins and fats and low in fibers, not only increases the insulin-like growth factor 1 levels that augment cancer risk but also shapes gut microbiota enriching the proinflammatory *Bacteroides* and *Enterobacteria*, while decreasing *Bifidobacteria*, *Eubacteria* and *Lactobacilli* [150–152].

Carbohydrates are the main carbon and energy source for gut microbes [153] and are among the most studied dietary components regarding their ability to modify the microbiota [154]. Microbes have the ability to transform dietary components and to provide important metabolic bioproducts, among which a growing body of interest is being devoted to short chain fatty acids (SCFAs). The latter are the end product of the fermentation of dietary fibers, with acetate, propionate and butyrate being the most abundant [155]. SCFAs, and especially butyrate, represent a fundamental energy source for colonic epithelium and also play important roles in the regulation of host lipid and glucose metabolism and in immune functions [156].

In our previous *in vivo* study, we demonstrated that replacement of digestible carbohydrates with non-digestible ones, within the diet of PC-induced mice, significantly reduces proinflammatory microorganisms (such as *E. coli*, *R. gnavus*, *B. acidifaciens* and *C. cocleatum*) and, on the other hand, increases levels of *Lachnospiraceae* and other butyrate-producing bacteria. This results in a decreased tumor volume [131]. Butyrate owns antineoplastic properties as it is able to interfere with cell proliferation, cell cycle, angiogenesis, inflammation and to enhance apoptosis [157]. Its derivative, phenylbutyrate (more stable and with a longer half-life), is under investigation in the clinical setting [158].

Lehouritis et al. [159] clearly demonstrated that local bacteria influence the efficacy of chemotherapeutic drugs, either by inhibiting or by improving efficacy. Specifically, *E. coli* was found to inhibit the gemcitabine effect when tested *in vitro* and in an *in vivo* mouse model of subcutaneous CRC, as demonstrated by the decreased survival and the increased tumor volume of mice treated with gemcitabine together with *E. coli* [159].

If it is true that microbiota composition can influence the response to anticancer drugs, it is nonetheless documented that pharmacological treatments in their turn can select certain microbial populations, thus influencing the course of the disease [160–162]. Unpublished data from our laboratory showed that in xenografted PC mice

subjected to gemcitabine treatment, the proportion of the Gram-positive *Firmicutes* and the Gram-negative *Bacteroidetes*, which are the two dominant phyla in the gut of tumor-bearing mice, decreased considerably as compared to control mice. Concomitantly, in the gut of drug-receiving mice, *Proteobacteria* and *Verrucomicrobia* became the most represented phyla. These and other alterations, observed at lower taxonomic levels, suggested us that gemcitabine treatment may select an inflammatory bacterial community, which may cause adverse reactions and may affect the clinical outcome.

Therefore, understanding the effect of chemotherapy on the modulation of gut microbiota may explain chemoresistance processes, thus helping to set up strategies to improve the effectiveness of therapy.

One of the main side effect of cancer and anticancer therapies is cachexia, a condition of skeletal muscle wasting and loss of lean body mass [163] accompanied by a state of systemic inflammation [163, 164]. Cachexia is even more frequent in the frame of gastrointestinal cancers [165] and is strictly associated with a poor response to therapeutics agents and with higher morbidity and mortality [163]. The therapeutic interventions applied to reverse cachexia are mainly based on pharmaconutritional support, focusing on palliation of symptoms and reduction of distress, but in many cases cachexia remains untreated [163]. A recent study revealed an altered composition of gut microbiota in two mouse models of cancer cachexia, both characterized by *Enterobacteriaceae* increased and *Lactobacillus* decreased [166]. Administration of a mixture of *Lactobacillus reuteri* and *Lactobacilllus gasseri* to leukaemic mice with cachexia was found to alleviate inflammation and partially rescue muscle from atrophy [167]. Similarly, a beneficial effect of the microorganism *L. reuteri* has been more recently demonstrated in ApcMIN mice with colon cancer and predisposed to cachexia: mice fed with *L. reuteri* in drinking water showed larger gastrocnemius muscle masses and a greater body weight as compared to untreated mice, together with reduced neutrophil counts, a marker of systemic inflammation [164]. Moreover, it was observed that administration of pectic oligosaccharides to leukaemic mice increased the abundance of *Bacteroides dorei*, alleviating the cachetic phenotype [168], and similar results were obtained administering a synbiotic mixture of *L. reuteri* and short-chain inulin-type fructans, with the concurrent reduction of leukaemic cells and prolonged survival [166].

Taken together, these results demonstrate that dietary interventions and supplementation of beneficial bacteria can reveal useful to restore eubiosis and positively guide the course of neoplastic diseases.

Conclusions

It is well known that intestinal microbiota can be easily manipulated through the diet. Certain foods selectively enrich some microbial groups, which in turn can shape the profile of the whole gut microbiota, thus affecting the onset and the progression of several diseases, including cancer. Proinflammatory microorganisms such as *B. acidifaciens*, *E. coli*, *R. gnavus* and *C. cocleatum* significantly decrease upon fiber-rich food regimens [131], substantiating the hypothesis that engineered diets able to perturb gut microbial community may synergistically interact with the current therapies.

Different interventions have been proposed as a tool to shape the gut microbiota in order to interfere with cancer progression, improve response to treatment or limit toxic side effects. In this regard, the most common approaches are represented by the administration of probiotics and prebiotics.

Preclinical studies suggesting that microbiota manipulation provides an opportunity to favorably change cancer progression and improve patients' survival already exist [121], but the heterogeneity in describing the different organs and substrates utilized in the different studies (salivary, tissue, serum or stool) together with the different methods used (16s DNA sequencing, quantitative PCR, ELISA detection or bacterial culture methods) call for standardization of the exploring methods.

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