Microbiota dysbiosis: a new piece in the understanding of the carcinogenesis puzzle

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Cancer is defined as an uncontrolled proliferation of malignant cells in a host and it is one of the main causes of death worldwide. Genetic and environmental factors play an important role in its development, and the involvement of microbial communities has also recently been recognized. The close relationship that characterizes the colonization by human commensal communities involves health risks, particularly when the homeostasis is disturbed. It has been hypothesized that this process may lead to cancer by modulating the inflammatory response of the host, by the production of carcinogenic metabolic products or by the production of toxins, which disrupt the cell cycle. The metabolic effects of the intestinal microbiota have been studied in greater detail in the gastrointestinal tract, and it has been recognized that microbial communities of other body surfaces can cause effects either locally or at a distance. In vitro and in vivo studies have allowed the characterization of the microbiota and the establishment of a cause and effect relationship with some types of cancer. Nevertheless, despite the results, representative studies are necessary to validate the findings and definitively establish the role of microbiota in cancer development in order to open the possibility of promising therapeutic and diagnostic applications. Thus, the aims of this review are to briefly examine the available evidence, and to analyse the mechanisms described for pancreatic, lung, colorectal cancer, oral squamous cell carcinoma and hepatocellular carcinoma and the impact of the current knowledge about the effects of the microbiota on carcinogenesis.

Introduction

Diseases date back to the dawn of humanity, with devastating plagues and infectious diseases accompanying mankind throughout its history on Earth; some have been eradicated, while others remain a problem of global concern. Improved public health, education and medical advances have significantly reduced death and disease in many parts of the world (Barrett et al., 1998; Morens & Fauci, 2013). However, the emergence of new ailments and globally prevalent chronic diseases (non-communicable diseases and conditions), such as allergies, asthma, coronary heart disease and cancer, play an important role in the post-modern era (Blaser, 2006; Dietert & Dietert, 2015).

Cancer is one of the primary causes of global death. According to the World Health Organization (WHO) projections, there will be more than 21 million new cases of cancer and more than 13 million cancer-related deaths worldwide by 2030 (Zong et al., 2012).

There are six essential alterations related to cancer in cell physiology: self-sufficiency in growth signals, insensitivity to growth inhibitory signals, evasion of apoptosis, unlimited proliferative capacity, angiogenesis and metastasis (Plottel & Blaser, 2011; Vineis et al., 2010; Zong et al., 2012). Cancer is the result of mutations in the chromosomal DNA of the cell; fewer than 10% of cases are caused by mutations in the germ line, the remaining are the result of acquired somatic mutations (Elinav et al., 2013; Zong et al., 2012). Disease and death are the results of metabolic and systemic effects.

Abbreviations: COPD, chronic obstructive pulmonary disease; CRC, colorectal cancer; DCA, dichloroacetic acid; HCC, hepatocellular carcinoma; HOMIM, human oral microbe identification microarray; IARC, International Agency for Research on Cancer; IBD, inflammatory bowel disease; LCA, lithocholic acid; LPS, Lipopolysaccharide; NGS, Next generation sequencing; NLRP6, NOD-like receptor family pyrin domain containing 6; NOD, nucleotide-binding oligomerization domain; OSCC, oral squamous cell carcinoma; PAHO, Panamerican Health Organization; PAMPS, pathogen-associated molecular patterns; PRR, pattern recognition receptor; ROS, reactive oxygen species; STAT3, signal transducer and activator of transcription 3; TLR, Toll-like receptor; WHO, World Health Organization.
of local and distant malignant cells (Plottel & Blaser, 2011). While genetic predisposition (hereditary mutations) has been recognized, host factors (hormones, immune conditions) and environmental factors (tobacco, alcohol, chemicals, infectious agents and radiation) have also been determined to pose a risk for cancer development (Plottel & Blaser, 2011; Zong et al., 2012). It is only recently that the importance of the microbial communities (i.e. bacteria, archaea, eukaryotes, viruses) that live in our bodies has been acknowledged (Clemente et al., 2012; Elina et al., 2013; Hassan et al., 2013; Plottel & Blaser, 2011).

It is debatable whether cancer is the product of variations in the microbiota, or whether modifications in the normal microbiome are the result of cancer progression; there is strong evidence of both (Blumberg & Powrie, 2012; Francescone et al., 2014). On the one hand, tumour cells exhibit differential profiles when compared with untransformed progenitors, and this may favour the selective adherence of some members of the microflora. Also, the hypoxic environment and nutrient availability in solid tumours can favour organisms that require low oxygen tension. On the other hand, there is growing evidence that disruption of microbial communities colonizing specific organs is directly or indirectly associated with carcinogenesis (Garrett, 2015; Khan et al., 2012).

According to Koch’s postulate, the classical concept involves the relationship ‘one microorganism – one disease’ (Clemente et al., 2012). However, after determining that the number of microorganisms (viruses, eukaryotes and bacteria) colonizing humans is extremely large, this concept was shown to be an oversimplification, and that it cannot explain the aetiology of a disease (Sheflin et al., 2014). From the $3.7 \times 10^{16}$ microbial inhabitants, only 10% have been categorized as carcinogenic for humans by the International Agency for Research on Cancer (IARC) (Garrett, 2015). It is known that some pathogens, such as Helicobacter pylori, promote the development of gastric cancer through epithelial damage and inflammation (Petersen & Round, 2014). Other microorganisms, such as viruses, cause cancer through well-described genetic mechanisms, as in the case of human papillomavirus, hepatitis B virus, hepatitis C virus, human herpesvirus 8 and human T-lymphotropic virus 1 (Bultman, 2014; Hassan et al., 2013; Schwabe & Jobin, 2013). However, the role of microorganisms in the initiation and progression of cancer cannot be simply described as a pathogen-related disease (Sheflin et al., 2014). Defects in the host’s regulatory circuits and alterations in the microbiota can change the symbiotic relationship and promote disease (Schwabe & Jobin, 2013). New insights have shown that some diseases are a product of the loss of balance in the commensal community, termed dysbiosis, rather than the presence of a single causative organism (Clemente et al., 2012; Petersen & Round, 2014).

The number of microbial cells that colonize the human body is $>10^{14}$, this is 10 times more than the total sum of human somatic and germ cells (Blaser, 2006; Bultman, 2014; Clemente et al., 2012; Petersen & Round, 2014; Plottel & Blaser, 2011). ’Microbiota’ is a collective term that refers to the group of microbes including bacteria, fungi, parasites and viruses colonizing the human body (Hassan et al., 2013; Plottel & Blaser, 2011), and the collection of genes they encode is known as our microbiome (Clemente et al., 2012). The microbiome, which is considered the ‘forgotten organ’ (Clemente et al., 2012) and our ‘second genome’ (Dietert & Dietert, 2015), contains a large number of genes that surpass the human genome in number by 100 times and perform key functions relevant for human health (Arslan, 2014; Schwabe & Jobin, 2013). The microbiome participates in the metabolism of nutrients and other environmental factors (Dietert & Silbergeld, 2015), vitamin synthesis (D’Argenio & Salvatore, 2015), inhibition of pathogenic growth (Kamada & Núñez, 2013), and maturation and maintenance of the immunological system (Schippa & Conte, 2014), while also ensuring a balance or homeostasis in the body (Arslan, 2014; Costello et al., 2009; Hooper & Macpherson, 2010; Schwabe & Jobin, 2013), stimulation of angiogenesis and regulation of host fat storage (Palmer et al., 2007).

The microorganism–human symbiotic interaction has been achieved after millions of years of co-evolution, co-adaptation and co-dependence (Blaser, 2006; Blaser & Falkow, 2009), having important consequences for human health and physiology (Hooper & Macpherson, 2010; Palmer et al., 2007). It is clear that this list of functions relevant to human health is not yet complete; as this field of study expands, we are continually discovering new roles and relationships (Palmer et al., 2007).

Microbiotic colonization begins at the moment of birth, progressing through the early stages of childhood (Blaser, 2006; Blaser & Falkow, 2009; Clemente et al., 2012; Plottel & Blaser, 2011). During this development, the association of microorganisms with humans is a non-random process that adapts to the body habitat (Khan et al., 2012; Turnbaugh et al., 2007). Today, it is known that the composition of microbial communities varies across different anatomical sites (Clemente et al., 2012; Costello et al., 2009; Hassan et al., 2013; Plottel & Blaser, 2011), depending upon the host’s genotype, physiological condition, lifestyle, environment and the presence of transient microorganisms (Turnbaugh et al., 2007; Walsh et al., 2014).

The great majority of microorganisms composing the human microbiota are bacteria residing within the gastrointestinal lumen (Walsh et al., 2014), existing in both competition and collaboration with other microorganisms in this niche (Kamada & Núñez, 2013). Thus, the environmental conditions within the gastrointestinal tract are a very dynamic product of specific intra individual interactions, influenced by diet, the rapid flow of nutrients, the presence of the host’s immune system, occasional infections and the use of antibiotics and chemotherapeutics (Belizário & Napolitano, 2015; Turnbaugh et al., 2007).
This close relationship can represent hazards for health (Hooper & Macpherson, 2010), particularly when the homeostasis is disturbed. Despite significant progress, the microorganisms that constitute what might be considered a 'normal or healthy microbiota' have not been clearly defined (Clemente et al., 2012; Rajilic-Stojanovic, 2013). However, patterns emerge when microbial communities are compared at higher taxonomic levels through molecular profiling (Hooper & Macpherson, 2010) between groups of ‘healthy individuals’ and cohorts of patients with different pathologies, such as inflammatory bowel disease (IBD), diabetes mellitus, obesity, cardiovascular disease and cancer (Bultman, 2014; Dietert & Dietert, 2015; Petersen & Round, 2014).

For decades, the study of the composition of microbial communities was based on the characterization of culturable microorganisms (Hooper & Macpherson, 2010); the development of molecular and bioinformatics techniques has allowed a better approach and greater comprehension of the microbiota (Blaser & Falkow, 2009). With the gradual advances in the field of microbial ecology, it may be possible to use the microbiota and its variations to understand human diseases, such as cancer (Bultman, 2014).

Understanding of the diverse contributions of the bacterial microbiota to carcinogenesis may provide opportunities for the development of effective diagnostic and preventive methods (Hassan et al., 2013; Ohtani, 2015; Plottel & Blaser, 2011). However, there are existing variables and challenges requiring further investigation (Rajilic-Stojanovic, 2013). The composition of the microbial communities colonizing humans is highly individualistic and it depends on multiple external factors (Khurana, 2012). For this reason, integrative studies of metagenomics, metatranscriptomics and metabolomics of a large cohort of patients and healthy controls are necessary to definitively establish the role of the microbiome in cancer development (Aguir-Pulido et al., 2016; Guinane & Cotter, 2013; Schwabe & Jobin, 2013).

Dysbiosis and carcinogenesis

Dysbiosis, defined as a disturbance in the microbiome structure (Belizario & Napolitano, 2015; Petersen & Round, 2014; Rajilic-Stojanovic, 2013), may consist of processes not necessarily mutually exclusive, such as loss of beneficial microorganisms, expansion of pathobionts or harmful microorganisms, and general loss of microbial diversity (Petersen & Round, 2014). Thus, it is possible that defects in the regulatory circuits of the host controlling homeostasis can disturb the symbiotic relationship and promote disease (Petersen & Round, 2014). Three types of relationships can be envisaged between the microbiome and mechanisms that give rise to cancers. In Class A, the primary interactions involve immunocytes; in Class B, the primary interactions involve local parenchymal cells; and in Class C, the local interactions produce distant effects (Plottel & Blaser, 2011).

Some types of bacteria are able to stimulate mediators of inflammation, producing toxins that disrupt cell cycle control or contribute to the tumorigenic process through metabolites (Bultman, 2014; Garrett, 2015; Hassan et al., 2013; Schwabe & Jobin, 2013). The following describes the mechanisms of carcinogenesis that are mediated by commensal microorganisms.

Mediated by the immune system effect

The human body harbours a vast number of commensal microbial species. The immune system plays an important role in the preservation of the symbiotic nature of the host–microbiota interaction and for this purpose it employs several strategies (Belizario & Napolitano, 2015; Hooper & Macpherson, 2010). In healthy mammals, commensal bacteria are primarily found in the intestinal lumen, with some bacteria being associated with the epithelium. There is an anatomical separation of commensal bacteria and cells through a system of physical and chemical barriers that prevent translocation to the underlying connective tissue (Fung et al., 2014; Hooper et al., 2012; Schwabe & Jobin, 2013). Thus, the loss of the natural barrier either physically (tight junctions, the mucous layer) or at the level of antibacterial defence systems (Schwabe & Jobin, 2013) enables access of cellular components (such as LPS) or microorganisms to systemic circulation where they may trigger an inflammatory response mediated by the innate immune system (Logan et al., 2016). This loss of the barrier may result from primary defects in genes encoding proteins essential for the maintenance of a functional barrier or from secondary defects due to infection or inflammation (Garrett, 2013; Schwabe & Jobin, 2013). Disturbed homeostasis produced by dysbiosis can favour pro-inflammatory or immunosuppressive responses with deleterious effects for the host (Belizario & Napolitano, 2015; Fung et al., 2014; Garrett, 2015).

Different cells (e.g. intestinal epithelial cells, haematopoietic cells) express pattern recognition receptors (PRRs) that mediate the interaction between the immune system and the commensal microbiota, such as Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors. These structures recognize microbes-associated molecular patterns such as LPS, peptidoglycan, flagella and microbial DNA/RNA. This observation supports the ‘cornerstone of innate immunity’ (Belizario & Napolitano, 2015; Schwabe & Jobin, 2013). Pro-inflammatory processes mediated by pathogen-associated molecular patterns (PAMPs) activate PRRs in various types of cells such as macrophages, myofibroblasts and epithelial cells that produce pro-inflammatory cytokines (IL-17, TNFα, IL-6, IL-β). The TLR signals induce survival intracellular pathways mediated by activation of NFκB and signal transducer and activator of transcription 3 (STAT3) (Arslan, 2014; Belizario & Napolitano, 2015; Schwabe & Jobin, 2013). The TLRs can also induce tumour proliferation mediated by mitogens such as epiregulin, amphiregulin and hepatocyte growth factors. This can occur locally or have an effect on other distant organs (Schwabe & Jobin, 2013).
Many studies have focused on TLR2, TLR4, NOD2 (muramyldipeptide), NLRP6 (NOD-like receptor family pyrin domain containing 6) and NOD1. These receptors have been shown to be relevant in the formation of tumours in murine models and polymorphisms related to human diseases, such as colorectal cancer (CRC) (Garrett, 2015; Schwabe & Jobin, 2013). For example, mice deficient in NLRP3 and NLRP6 show an increased susceptibility to CRC due to the absence of the protective cytokine IL-18 (Elinav et al., 2013). The overexpression of TLR molecules has been observed in individuals who have developed oral squamous cell carcinoma (OSCC) (TLR3, TLR4, TLR7 and TLR9) or lung cancer (TLR4 and TLR9) when compared with normal cells (Khan et al., 2012).

Generally, more than 20% of cancers are preceded by chronic inflammation, such as in hepatocellular carcinoma (HCC) and CRC (Francescone et al., 2014). A distinctive characteristic of inflammation is the activation of cellular components that participate in tumorigenesis (Elinav et al., 2013). However, the role of inflammation is not limited to initiation, but is also relevant to tumour growth (Francescone et al., 2014). Tumour-infiltrating myeloid cells produce proinflammatory cytokines, such as IL-6, that induce STAT3 and NRκB signalling to increase cellular proliferation and to suppress apoptosis (Elinav et al., 2013). STAT3 is commonly implicated in the tumorigenesis of several tissues, and in the inflammatory process of liver, lung, pancreas and colon cancers (Elinav et al., 2013).

**Genotoxic effect**

A genotoxic agent is one that produces direct DNA damage, has mutagenic properties and usually participates in the stages of cancer initiation or progression (Klaunig & Kamendulis, 2010).

Inflammation is recognized to play a role in tumorigenesis by increasing DNA damage while compromising the mechanism of repair, leading to instability (Elinav et al., 2013). Reactive oxygen species (ROS) are released by macrophages in response to pro-inflammatory cytokines, causing oxidative damage to macromolecules, DNA breakage, mutations, and damage and stimulation of pathways leading to the activation of transcription factors, such as NRF2 and NFκB, that modulate cellular growth to produce cancer (Francescone et al., 2014; Klaunig & Kamendulis, 2010).

Furthermore, many microorganisms have developed survival and competition mechanisms, such as proteins that damage DNA. These proteins lead to mutational effects in the host because they favour proliferative signalling, affect genome stability and resist cellular death (Garrett, 2015). Among them we can mention *Enterococcus faecalis*, *Bacteroides fragilis*, *Escherichia coli*, *Fusobacterium nucleatum* (see Table 1) (Francescone et al., 2014; Ohtani, 2015; Schwabe & Jobin, 2013; Sheflin et al., 2014).

**Metabolic effect**

The human metabolism represents a combination of human and microbial enzymic activities, with the bacterial metagenome playing an important role in the biosynthesis and metabolism of various compounds. As mentioned by Turnbaugh et al. (2007): ‘If we consider ourselves to be a composite of microbial and human species, our genetic landscape a summation of the genes embedded in our human genome and microbiome, and our metabolic features a coalescence of human and microbial traits, the self-portrait that emerges is one of a “human supraorganism”’.

### Table 1. Bacterial genotoxins

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Producing microorganism</th>
<th>Mechanism/effect</th>
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<tbody>
<tr>
<td>FadA</td>
<td><em>Fusobacterium nucleatum</em></td>
<td>Adhesin present on bacterial surface and involved in invasion. Binds to E-cadherin in CRC cells activating β-catenin, leading to an oncogenic and inflammatory response with proliferation of tumour cells (Garrett, 2015; Schwabe &amp; Jobin, 2013; Rubinstein et al., 2013).</td>
</tr>
<tr>
<td>Btf</td>
<td><em>Enterotoxigenic Bacteroides fragilis</em></td>
<td>Activates β-catenin through E-cadherin. Can indirectly damage DNA by increasing ROS levels. In <em>in vitro</em> and <em>in vivo</em> models, this induces an increase of cell proliferation and release of pro-inflammatory mediators (such as IL-17) (Boleij et al., 2015; Garrett, 2015).</td>
</tr>
<tr>
<td>AvrA</td>
<td><em>Salmonella typhi</em></td>
<td>Has been related to chronic infection and hepatobiliar cancer. Induces cell proliferation, acquisition of stem-cell-like qualities and loss of cell polarity by activation of β-catenin (Garrett, 2015).</td>
</tr>
<tr>
<td>Cdt (cytotoxic distending toxin)</td>
<td>Several <em>Epsilonproteobacteria</em> and <em>Gammaproteobacteria</em></td>
<td>Produces genomic instability and triggers responses to dsDNA damage leading to cell cycle arrest and cell swelling (Gagniére et al., 2016; Garrett, 2015).</td>
</tr>
<tr>
<td>Colibactin</td>
<td>E2 group <em>Escherichia coli</em></td>
<td>Leads to dsDNA rupture contributing to inflammation-mediated CRC (Vizcaino &amp; Crawford, 2015).</td>
</tr>
<tr>
<td>CagA</td>
<td><em>Helicobacter pylori</em></td>
<td>Activates β-catenin leading to an increase in cell proliferation and plays an important role in the progression of gastric malignancy (Garrett, 2015).</td>
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</tbody>
</table>
As the intestinal microbiota is the most studied, and currently shown to have the most recognized impact on human health, study of the metabolic activity of this microbiota can lead to increased understanding of toxic products, such as acetaldehyde originating from the conversion of alcohol (Khurana, 2012), nitrosamines and other products of protein fermentation, oestrogen deconjugation and generation of secondary bile acids, resulting in the metabolic activation of carcinogens (Ohtani, 2015; Schwabe & Jobin, 2013).

**Cause and effect relationship**

Traditional culturing methods enable the isolation of less than 30% of the human bacterial microbiota. These methods have limitations and do not represent the actual microbial community (Guinane & Cotter, 2013; Schwabe & Jobin, 2013). Metagenomic and culturomics techniques which include gene sequencing, microarrays and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-ToF/MS) produce a more accurate characterization of the microbiota (Greub, 2012; Guinane & Cotter, 2013). With regards to advances in sequencing technologies, decreased cost and the consequent development of metagenomic projects, transcriptomics, metabolomics and powerful software applications, have supported major advances in the understanding of the human microbiome, expanding our horizon on microbial species colonizing the human body and their relationship to health and disease (Belizário & Napolitano, 2015; Hooper et al., 2012; Wang & Ganly, 2014).

In **vivo** and **in vitro** investigation models are used to characterize the role of microbiota in carcinogenesis and to establish their cause and effect, thereby facilitating both the manipulation of variables and the exploration of metagenomic-derived hypotheses. The **in vivo** models include animals either maintained in a germ-free state or colonized with specific bacteria (gnotobiotics) (Bultman, 2014; Turnbaugh et al., 2007). Colonization of these animals with different species or bacterial groups enables the study of the association of these bacteria with a particular function at various stages of the animals’ life cycles (Turnbaugh et al., 2007) and thereby their microorganism–host interactions (Palmer et al., 2007) or, more specifically, immunological response. Zebrafish, mice, rats and even pigs have been successfully used, with mice being the most widely used and characterized (Fritz et al., 2013).

Another approach to study the microbiota–host relationship is the use of conventionally raised animals treated with antibiotics, supplemented with a special diet, or with deletions in the genes that mediate a pro-inflammatory immune response, which leads to inhibition or proliferation of certain bacterial communities. For example, changes in the immune response have been observed in Il10−/−, Nod2−/−, Asc−/− and Nlrp6−/− rats (Bultman, 2014; Khurana, 2012; Petersen & Round, 2014; Schwabe & Jobin, 2013).

The **in vitro** models reflect the processes that occur in certain organs, such as the simulator of the human microbial ecosystem, SHIME, which uses different serially connected bioreactors or micro-channels (Turnbaugh et al., 2007). These **in vitro** models allow analysis of metabolic processes in microbial digestion of certain compounds in order to understand the pharmacokinetics of certain drugs (Fritz et al., 2013) and for the study of microbiota variations that are influenced by diverse factors. The **in vivo** models include cultured cells that resemble the architecture of tissue (Fritz et al., 2013).

Despite the progression in research to better understand microbial species associated with disease, existing studies are not sufficiently conclusive or statistically significant, due to both the number of cases studied and the heterogeneity of the microbiota that is influenced by many external factors (Fritz et al., 2013; Schwabe & Jobin, 2013).

**Types of cancer that are considered related to microbiome dysbiosis**

The gastrointestinal tract is one of the most extensive compartments in the human body (~250 m²) and harbours the largest number of microorganisms colonizing humans (Khurana, 2012). As such, it has the foremost metabolic effect of all microbiomes. As the most frequently investigated organ system, the gastrointestinal tract has been used as a model for understanding the host–microbiota and disease relationship (Pevsner-Fischer et al., 2016; Schwabe & Jobin, 2013). Other organs, such as the skin, oral cavity and female genital tract, that also contain a considerable number of microorganisms have been less frequently studied (Schwabe & Jobin, 2013). Growing evidence supports a key role for bacterial microbiota in carcinogenesis of the colon, liver (Cesaro et al., 2011; Darnaud et al., 2013; Hines et al., 2010; Roh & Seki, 2013), lung, oral cavity and pancreas (Michaud et al., 2007; Ochi et al., 2012; Schwabe & Jobin, 2013).

**Hepatocellular carcinoma (HCC)**

The Panamerican Health Organization (PAHO), jointly with the WHO, have estimated that 78 126 new cases of HCC will be diagnosed in the Americas in 2020, with 49 763 new cases among men and 28 363 among women (Ferlay et al., 2013, IARC), with an estimated 72 437 people expected to die of this disease by that date. HCC is one of the most frequently diagnosed human cancers worldwide, with 80–90% of cases being preceded by chronic liver disease, liver fibrosis and cirrhosis (Roh & Seki, 2013). Most HCC cases have developed as the result of chronic hepatic inflammation (Fung et al., 2014; Tsiaoussis et al., 2015). Chronic liver disease is the result of several aetiologies, from alcohol consumption to viral infection, which are related to the increased risk of fibrosis, cirrhosis and liver failure (Fung et al., 2014; Roh & Seki, 2013).
The liver receives 70% of its blood supply from the intestinal venous blood flow, and due to this close functional relationship between the liver and the gastrointestinal tract (gut–liver axis), there is constant exposure to nutrients, toxins, antigens derived from food, microbial products and microorganisms (Arslan, 2014; Hines et al., 2010; Ohtani, 2015). This organ is equipped with a wide repertoire of immune cells (macrophages, lymphocytes, natural killer cells, dendritic cells) whose function is to present the first line of defense against antigens derived from the intestine (Liaskou et al., 2012; Cesaro et al., 2011). The potential role of the intestinal microbiota in liver diseases has been known since 1921 (Arslan, 2014). The microbiome of the liver, although not fully understood, provides an example of how cancer may be promoted by dysbiotic microbiota through long-distance mechanisms (Schwabe & Jobin, 2013).

The relationship between the loss of intestinal barrier function and liver damage has been studied (Hines et al., 2010); immune cells (Kupffer cells and hepatic stellate cells) and their interaction with bacterial products infiltrating from the intestine contribute to the general damage (Minemura & Shimizu, 2015). It has been proposed that products derived from microorganisms, such as LPS, participate in the progression of HCC through the release of pro-inflammatory cytokines (Hines et al., 2010). Increased intestinal permeability, bacterial translocation and LPS accumulation activating the NFκB pathway suggest a hallmark of chronic liver disease and contribute to hepatic inflammation, pro-inflammatory cytokines TNF-α, IL-6 and IL-1 release, oxidative damage and fibrosis (Tsiaoussis et al., 2015).

Direct manipulation of the microbial community in germ-free, gnotobiotic and/or antibiotic-treated mice has revealed the role of the microbiota in HCC. While the microbiota is not necessary for initiation, it does support the development of HCC (experimental evidence is shown in Table 2) (Darnaud et al., 2013; Hines et al., 2010; Roh & Seki, 2013). Intestinal bacteria can promote liver cancer through pro-inflammatory PAMPS and bacterial metabolites that reach the liver via the portal vein. Increased intestinal bacterial translocation contributes to inflammation and fibrosis in chronic liver disease. Hepatocarcinogenesis occurs in a chronically injured organ and depends on the activation of TLR4 (Dapito et al., 2012), which increases the proliferative effect of mitogens such as epiregulin and decreases apoptosis (Darnaud et al., 2013; Hines et al., 2010; Roh & Seki, 2013). The administration of antibiotics in late stages of disease reduces the microbiota TLR4 anti-apoptotic signalling effect in HCC (Elinav et al., 2013). The role of other TLRs, such as TLR2 and TLR9, in chronic liver disease remains to be determined (Roh & Seki, 2013).

Studies in animal models (murine) have shown that the enterohepatic circulation of dichloroacetic acid (DCA) provokes DNA damage and consequent cellular senescence in hepatic stellate cells which, in turn, secrete various inflammatory and tumour-promoting factors in the liver, thus facilitating HCC (Elinav et al., 2013; Hara, 2015; Ohtani, 2015). Another mechanism suggested is the conversion of ethanol into toxic and carcinogenic acetaldehyde by anaerobic bacteria in the colon. Therefore, ethanol can be attributed to the functional disruption of the intestinal barrier allowing the flux of endotoxins and mediators which may be involved in the aetiology of hepatic disease (Ohtani, 2015).

**Pancreatic cancer**

The PAHO/WHO have estimated that, in 2020, 94,625 new cases of pancreatic cancer will be diagnosed in the Americas, with an almost even distribution of 46,913 new cases among women and 47,712 among men (Ferlay et al., 2013, IARC). Furthermore, an estimated 93,283 people are expected to die of this disease. Pancreatic cancer is an aggressive malignancy, with a low rate of survival and therapeutic success (Mitsuhashi et al., 2015; Pevsner-Fischer et al., 2016). Therefore, it is important to further understand its pathogenesis and to search for new diagnostic and therapeutic alternatives (Mitsuhashi et al., 2015; Zambirinis et al., 2014).

Inflammation is clearly a critical factor in pancreatic carcinogenesis, and the microbiome has been associated with inflammation-initiated cancer. Although not yet well described, the most readily accepted mechanism is the activation of the immune system, and the perpetuation of inflammation associated with the tumour, rather than direct mutagenicity (Zambirinis et al., 2014). As with the liver, the pancreas does not present a known microbiome, but it is presumed that carcinogenesis in this organ is supported by distant dysbiotic microbiota (Schwabe & Jobin, 2013).

Recently, periodontal disease and limited oral hygiene have been recognized as risk factors for pancreatic cancer, as they facilitate the translocation of bacteria (Zambirinis et al., 2014). As reported by Mitsuhashi et al. (2015), bacteria can reach the pancreas through circulation, and it has been suggested that this occurs through the biliary tract (transductal transmission) (Zambirinis et al., 2014) to act synergistically with other risk factors, such as obesity and smoking, among others, during carcinogenesis.

It has been established that the oral cavity is a large reservoir of bacteria, comprising over 700 species or phylotypes, with pathological evidence in other organs. The human oral microbe identification microarray (HOMIM) profile of salivary microbiota reveals significant variations in the composition of the microbiota between pancreatic cancer patients and healthy controls (Bultman, 2014). Prospective studies have shown an association between periodontal disease and an increased risk of developing pancreatic cancer. There is also an association between the oral microbiome and periodontitis in pancreatic carcinogenesis (Farrell et al., 2012; Michaud et al., 2007).

Disturbances of the intestinal permeability produce environmental injuries that allow the access of pathobionts into circulation. One of the most relevant examples is the consumption of alcohol, which is one of the primary causes of
Table 2. Experimental evidence of relationship between microbiota and HCC

<table>
<thead>
<tr>
<th>Authors (year)</th>
<th>Variables</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yu et al. (2010)</td>
<td>Diethylnitrosamine (DEN) injection in C57BL/6 mice for intestinal sterilization/TLR4 deletion.</td>
<td>HCC was induced with lower incidence in TLR4 deficient (TLR4 -/-)/intestinal-sterilized mice</td>
</tr>
<tr>
<td>Dapito et al. (2012)</td>
<td>DEN/CCI4 intoxication of WT and mice sterilized with antibiotics/germ-free mice Administration of low doses of LPS</td>
<td>LPS–TLR4 pathway plays a role in promoting HCC in liver with chronic damage</td>
</tr>
<tr>
<td>Yoshimoto et al. (2013)</td>
<td>WT C57BL/6 mice, neonatal treatment with DMBA*; normal and high-fat diet p21-p-luc mice [inductor expression monitoring of secretory phenotype associated with senescence (SASP)] Dichloroacetic acid (DCA) production blockade or microbiota depletion by antibiotic treatment</td>
<td>Hepatocarcinogenesis depends on the TLR4 activation of resident cells in the liver not derived from bone marrow Role of intestinal bacteria in the development of obesity-associated HCC; mice with high-fat diet developed HCC, but not control mice The SASP produced by enterohepatic circulation of DCA has an important role in secreting inflammatory factors and tumour promoters</td>
</tr>
</tbody>
</table>

*DMBA, 7,12-dimethylbenz(a)anthracene.

chronic pancreatitis and is related to the dysfunction of the intestinal barrier and elevated levels of circulating LPS (Zambirinis et al., 2014). Recent studies suggest that inflammatory PAMPs, such as LPS and its receptor TLR4, are involved in pancreatic carcinogenesis (Schwabe & Jobin, 2013; Zambirinis et al., 2014). There is a possible role for endogenous LPS derived from intestinal bacteria in the modulation of pancreatic carcinogenesis (Ochi et al., 2012), although the mechanisms are unknown (Table 3). Upregulation of TLR7 has been observed to protect KC mice from pancreatic carcinogenesis (Zambirinis et al., 2014).

Lung cancer

Lungs are a surface with large exposure; as mentioned by Dickson et al. (2014): ‘the internal surface area of the lungs is 30 times that of the skin. Each day, this enormous terrain is exposed to more than 8000 liters of inhaled air, carried to within 0.1 micrometers of one tenth of the body’s total blood volume. The lungs are arguably the human body’s largest and most intimate interface with the outside environment and far from being a sterile surface (Dickson & Huffman, 2015). Lung cancer is an important cause of mortality worldwide. Although about 90% of cases are attributed to smoking, only 10–15% of smokers develop cancer, suggesting other influences, such as chronic lung inflammation (Houghton, 2013; Pevsner-Fischer et al., 2016).

Chronic obstructive pulmonary disease (COPD) is defined as the obstruction in the flow of air (Pragman et al., 2012). It comprises two components: airway obstruction and obliteration of small air sacs in the peripheral regions of the lung (emphysema) (Houghton, 2013). Numerous epidemiological studies have consistently linked the presence of COPD to the development of lung cancer (Mekkamu et al., 2013). The association between lung cancer and COPD has been attributed to the co-localization of genes that determine sensitivity to toxic pro-inflammatory and neoplasia-causing agents (Houghton, 2013).

Traditional microbial culture techniques have shown that approximately 50% of the exacerbations of COPD are associated with microorganisms such as *Streptococcus pneumo- niae*, *Haemophilus influenzae* and *Moraxella catarrhalis*. These organisms are often found colonizing the respiratory airways of patients with exacerbations of COPD. Many of these resistant bacteria are found in the airways of patients with COPD, and their presence can promote a chronic inflammatory state that promotes pathogenesis. Studies are not conclusive about microbial diversity in COPD patients compared with control subjects; data are inconsistent and poorly reproducible across studies employing different samples (tissue, bronchoalveolar lavage, sputum) (Sze et al., 2014).

Previous studies have shown that lung microbiota reflects the microaspiration of oral microbiota and is more diverse in the development of COPD versus control individuals. A recent study, in which surgical lung tissue samples were used, concluded that the microbiota of the lung is unique, with proteobacteria as the dominant genera (Yu et al., 2016).

Variations in the microbiota can induce a state of inflammation that accelerates the progress of COPD (Pragman et al., 2012). Although epidemiological evidence points to an association between COPD and lung cancer (Houghton, 2013; Schwabe & Jobin, 2013), the underlying molecular events are still not well known (Mekkamu et al., 2013). A study suggested that the commensal microbiota contributes to the response against lung cancer and that co-treatment with probiotics in the murine model can contribute to improving the pro-apoptotic and antiproliferative effects of cisplatin chemotherapy (Gui et al., 2015).

Squamous cell cancer

Squamous or squamous cell carcinoma is a malignant neoplasm that originates from squamous epithelial cells lining the mucosa and is most commonly found in the oral cavity...
The normal oral microbiome is composed of microorganisms which include a limited range of bacterial phyla: *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Actinobacteria* and *Fusobacteria*, with *Streptococcus*, *Prevotella*, *Veillonella*, *Neisseria* and *Haemophilus* being the predominant bacterial genera. The number of *Firmicutes* and *Actinobacteria* decreases in the presence of pre-cancerous and cancerous lesions, while the number of *Fusobacteria* increases (Schmidt et al., 2014; Wang & Ganly, 2014). Changes in relative abundance of the aforementioned variables demonstrate the differences between cancer patients and control patients (Schmidt et al., 2014). In 80% of cases of OSCC, between oral microbiota and malignant lesions due not only to differences in the microbiota between individuals, but also to differences in the microbiota between micro-environments within the oral cavity (lateral vs dorsal tongue, enamel surface, and so forth) (Dewhirst et al., 2010; Meurman, 2010).

Difficulties in establishing the relationship between the composition of the oral microbiota and malignancy could also be due to the limitations of earlier studies, restricted to the analysis of only a small number of oral bacterial species that can be cultured (Wang & Ganly, 2014), and it should also be kept in mind that optimal sampling of oral cancer patients may be difficult (Meurman, 2010).

The pathogenesis of OSCC is mainly attributed to the effects of smoking and heavy alcohol consumption. The oral cavity harbours one of the most diverse microbiomes in the human body, which includes viruses, fungi, protozoa, archaea and bacteria. There is increasing interest in the potential role of bacteria in this malignancy (Wang & Ganly, 2014).

Poor oral hygiene and periodontal disease have long been linked with premalignant lesions and carcinoma of the oral cavity. Periodontitis is a chronic inflammatory disease associated with biofilm-forming, anaerobic, Gram-negative bacteria leading to the liberation of inflammatory markers and bacteria into the saliva and at a low level into the blood, which may trigger systemic adverse reactions. The carcinogenic mechanisms, however, are not completely understood (Meurman, 2010; Tezal et al., 2009).

Changes in the composition of bacterial and fungal microbiota of the oral cavity and the association with oral cancer and pre-cancer have been reported. However, there is no consensus among existing reports associated with changes in the microbiome. It is difficult to establish the relationship between oral microbiota and malignant lesions due not only to differences in the microbiota between individuals, but also to differences in the microbiota between micro-environments within the oral cavity (lateral vs dorsal tongue, enamel surface, and so forth) (Dewhirst et al., 2010; Meurman, 2010).

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Table 4. Microbiota differences between normal individuals and OSCC patients

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Variables</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pushalkar et al. 2011;</td>
<td>454 parallel DNA sequencing, ~58,000 PCR amplicons that span the V4-V5</td>
<td>Members of 8 bacterial phyla were detected; most sequences</td>
</tr>
<tr>
<td>Wang &amp; Ganly 2014</td>
<td>hypervariable region of rRNAs from five subjects were sequenced (saliva</td>
<td>classified belonged to the phyla Firmicutes (45%) and Bacteroidetes</td>
</tr>
<tr>
<td></td>
<td>of 3 OSCC patients and that of 2 normal controls)</td>
<td>(25%); 15 unique phylotypes were present in all three OSCC subjects</td>
</tr>
<tr>
<td>Mager et al. 2005;</td>
<td>Saliva samples were collected from 229 OSCC-free and 45 OSCC subjects</td>
<td>High salivary counts of Capnocytophaga gingivalis, Prevotella melaninogenica and Streptococcus mitis may be diagnostic indicators of OSCC</td>
</tr>
<tr>
<td>Wang &amp; Ganly 2014</td>
<td>and evaluated for their content of 40 common oral bacteria using</td>
<td></td>
</tr>
<tr>
<td></td>
<td>checkerboard DNA–DNA hybridization</td>
<td></td>
</tr>
<tr>
<td>Nagy et al. 1998;</td>
<td>Biofilm samples were obtained from the central surface of the lesions in</td>
<td>V. alginolytica, F. nucleatum, P. melaninogenica, S. mitis, and S.</td>
</tr>
<tr>
<td>Wang &amp; Ganly 2014</td>
<td>21 OSCC patients (20 male, 1 female) aged 52.8 (±8.2) years, and from</td>
<td>melanolactica, P. melaninogenica, S. mitis, and F. nucleatum may be</td>
</tr>
<tr>
<td></td>
<td>contiguous healthy mucosa, before any antibiotic therapy or any tumour</td>
<td>diagnostic indicators of OSCC</td>
</tr>
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<td>treatment</td>
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</table>

oral bacteria Capnocytophaga gingivalis, Prevotella melaninogenica and Streptococcus mitis have been found to be present and are considered to be markers (Khurana, 2012). Some studies have shown differences in the oral microbiota between healthy individuals and patients with OSCC (Table 4).

The vast majority (95%) of the species/phylotypes identified in the OSCC samples represent oral taxa. While they are probably commensals adapting to the tumour tissue environment, the possibility that some may contribute to the development of OSCC or modify its clinical course cannot be excluded. Oral microorganisms inevitably up-regulate cytokines and other inflammatory mediators that affect the complex metabolic pathways and may thus be involved in carcinogenesis (Meurman, 2010). The role of highly abundant species, particularly those with pathogenic potential such as Prevotella oris, Aggregatibacter segnis and Fusobacterium spp., should be further explored, probably by testing them against oral epithelium in vitro; for example, the oral microbiota has been demonstrated to produce carcinogenic levels of acetaldehyde (Al-Hebshi et al., 2015). Although new molecular methods, such as next-generation sequencing, have greatly expanded our knowledge of the composition and function of the oral microbiome in health and disease, prospective studies will serve to resolve the temporal order between microbiome changes and the development of oral cancer (Wang & Ganly, 2014).

Colorectal cancer

Colorectal cancer (CRC) is one of the primary causes of death worldwide (Zackular et al., 2013). Ferlay et al. (2013) has determined that CRC is the third leading cancer, with 1.4 million new cases diagnosed in 2012. The PAHO/WHO have estimated that 305,968 new cases will be diagnosed in the Americas in 2020, with 156,813 cases among men and 149,155 among women, and an estimated 140,842 patients are expected to die of this disease by that date.

The sequence of mutational events that characterize the transition from normal colonic tissue to premalignant adenoma to invasive carcinoma has been well described (Kerr, 2003). The adenomatous polyposis coli (APC) gene encodes a protein involved in cellular adhesion and transcription. Mutations in this gene have been observed in 85% of CRC cases (Kerr, 2003; Louis et al., 2014). The KRAS (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homologue) gene codes for a GTPase that controls cellular proliferation and is mutated in 50–60% of CRC cases (Kerr, 2003; Louis et al., 2014). While genetic predisposition has been related to risk, clinical and experimental evidence also supports a relationship with diet and lifestyle (Greer & O’Keefe, 2011; Louis et al., 2014; Sheflin et al., 2014). Important risk factors such as the consumption of red and processed meats and alcohol, and chronic inflammation of the gastrointestinal tract (colitis-associated cancer in individuals with IBD) (Louis et al., 2014). A large body of evidence supports a relationship between infective agents and human cancers, and suggests that certain mucosa-associated bacterial species play an important role in the pathogenesis of CRC (Marchesi et al., 2011).

The first studies examining the relationship between the dominant microbiota and CRC incidence compared a Western diet, rich in meat and animal products, to a rural African diet rich in fibre, vegetables and grains (Greer & O’Keefe, 2011; Sheflin et al., 2014). Comparative epidemiological studies between populations with high and low CRC incidence have shown that, in addition to genetic factors, inflammation, infection, antimicrobial therapy and diet are also closely associated to risk for CRC development and are
related to changes in the diversity and activity of microorganisms that colonize the gastrointestinal tract (Arthur & Jobin, 2011; Louis et al., 2014; Zeller et al., 2014). Numerous studies have indicated that alterations in the microbial community occur rapidly after dietary changes, with characterization of the composition of the intestinal microbiota showing variances in the dominant phylum (Arthur & Jobin, 2011; Louis et al., 2014). The large intestine is colonized by a vast number of bacteria (approximately $10^{11}$–$10^{12}$ bacteria per gram of faeces) (Louis et al., 2014; Ridlon et al., 2006) belonging to few phyla. However, there is great diversity among the species present, with members of the phyla Firmicutes and Bacteroidetes being the most prevalent in healthy adults, and those of the phyla Proteobacteria, Actinobacteria, Fusobacterium, Verrucomicrobia and Cyanobacteria present to a lesser degree (Arthur & Jobin, 2011; Belizário & Napolitano, 2015; Palmer et al., 2007). This heterogeneous and metabolically active microbial community lives in symbiosis with the host, and it plays an important and active role in the metabolism of dietary constituents and in the modulation of the immune system (Greer & O’Keefe, 2011; Ridlon et al., 2006).

The identification of a ‘core microbiome’ in healthy individuals (Walsh et al., 2014) and the comparison with those found in patients and studies in experimental models may indicate the presence of potentially related dysbiotic microbiota in patients with CRC, as is already associated with IBD (Arthur & Jobin, 2011; Sheflin et al., 2014), autoimmune and allergic diseases, obesity and diabetes (Arslan, 2014; Clemente et al., 2012; Petersen & Round, 2014). In this regard, the murine microbiota is recognized to have a striking similarity with the intestinal human microbiota, and mouse model studies have provided an important understanding of the relationship between health and disease in humans. Changes in the composition of the gut microbiota have been found to be related with several clinical conditions such as obesity, diabetes, fatty liver disease, atherosclerosis, allergic diseases, gastrointestinal diseases, autoimmune diseases and cancer (Arslan, 2014).

Evidence regarding the contribution of the intestinal microbiota to the aetiology of pathological conditions, such as IBD and CRC, is increasing, with microorganisms belonging to the microbial community no longer simply being considered ‘spectators’ (Arthur & Jobin, 2011), but as metabolically active residents that strongly influence processes that maintain homeostasis or promote carcinogenesis (Zackular et al., 2013).

The microbiota may contribute to CRC through pro-carcinogenic activity of specific pathogens (Greer & O’Keefe, 2011). Studies on the role of PRR signalling and inflammatory responses and the metabolic capacity (metabolome) have been associated with activation of carcinogens that promote DNA damage and chromosome instability, causing an imbalance between cellular proliferation and apoptosis (Arthur & Jobin, 2011).

**Carcinogenic mechanisms associated with CRC**

**Harmful metabolites.** Certain dietary compounds have been associated with protective effects on the colonic mucosa. Short chain fatty acids (SCFA), such as acetate, propionate, butyrate and l-lactate, have potent anti-inflammatory, anti-proliferative and hence anti-neoplastic properties (Arslan, 2014; Garrett, 2015; Greer & O’Keefe, 2011; Louis et al., 2014; Petersen & Round, 2014; Rajilic-Stojanovic, 2013). However, there are metabolic products which, unlike SCFA, are detrimental to the health of the host and favour colonic carcinogenesis (Louis et al., 2014; Ohtani, 2015).

Metabolism in the colon occurs when undigested dietary components reach the large intestine and are fermented through anaerobic respiration. High protein intake is associated with an increase in colonic fermentation (Louis et al., 2014). Bacteroides and Firmicutes, among other bacteria, ferment aromatic amino acids which produce potentially bioactive products, such as phenylacetic acid, phenols, indoles and p-cresol (Dapito et al., 2012; Rajilic-Stojanovic, 2013; Schwabe & Jobin, 2013). Some products, particularly N-nitroso compounds, also produce nitrogen alkylation of DNA, which can induce mutations leading to carcinogenesis. Another product of protein fermentation is ammonium, which is potentially carcinogenic at low concentrations (Louis et al., 2014).

**Hydrogen sulfide.** In anaerobic respiration, nitrates, sulfates and organic compounds can act as electron acceptors. Microorganisms that use hydrogen and formate similar to methanogenic archaea and sulfate-reducing bacteria play an important role through cross-species interaction and alterations in the diversity of colonic bacteria that stimulates the growth of sulfur-reducing bacteria to compete for hydrogen. The methanogenic/archaea ratio varies in adults, and it may be important to determine the contribution of methanogenesis, acetogenesis and sulfate reduction. Sulfate-reducing bacteria are found in low concentrations in most individuals and are considered a co-substrate for growth and the formation of sulfide (Louis et al., 2014; Rajilic-Stojanovic, 2013).

Hydrogen sulfide is a product of sulfate reduction derived from the diet and metabolism of other compounds, including sulfur amino acids and taurine (Ridlon et al., 2006). Unlike methane, hydrogen sulfide is inflammatory, toxic for colonocytes, inhibits oxidation of butyrate, increases proliferation of colonocytes possibly through the ERK1/2 signalling pathway (Ridlon et al., 2006), inhibits the synthesis of mucus, affects cytochrome oxidase and is genotoxic through the generation of free radicals. It is believed that the mechanism of DNA damage may involve ROS (Louis et al., 2014).

**Secondary bile salts.** Secondary bile salts DGA, 3α,12α-dihydroxy-5b-cholan-24-oic acid and lithocholic acid (LCA; 3α-hydroxy-5b-cholan-24-oic acid) are products of
the microbial metabolism in the intestine (Ridlon et al., 2006). Primary changes in the large intestine include deconjugation, oxidation of hydroxy groups in C-3, C-7 and C-12, and 7α/β dehydroxylation. The deconjugation and 7α/β dehydroxylation of bile salts increases their hydrophobicity, allowing recovery through the colonic epithelium. Fat intake stimulates the synthesis and enterohepatic circulation of primary bile salts, which are reabsorbed and converted into the secondary salts, DCA and LCA (Greer & O’Keefe, 2011).

Recent evidence indicates that bacteria in Clostridium cluster IX may be a possible source of increased DCA and cancer risk in obese mice (Sheflin et al., 2014). DCA and LCA have been linked to carcinogenesis in several animal models and epidemiological studies, which concluded that DCA is a promoter of carcinogenesis. Cell signalling is activated by DCA in mammary epithelial cells through protein kinase C, and ERK1/2 through epidermal growth receptors. Secondary bile salts have also been shown to exert selective pressure for the emergence of mutations resistant to apoptosis (Ridlon et al., 2006). Moreover, the bile salts mediate damage to cell membranes through their amphipathic properties and exert antimicrobial activity to change the composition of the intestinal microbiota. They have also been associated with the disruption of membranes through the generation of ROS and reactive nitrogen species, which cause DNA damage by activation of membrane-associated proteins such as oxidase NADPH and phospholipase A2 (Louis et al., 2014).

Ethanol. Certain microorganisms have been demonstrated to oxidize ethanol into acetaldehyde, which is a compound that causes DNA damage and thus constitutes a major carcinogen (Louis et al., 2014).

Inflammation and dysbiosis. Colonic mucosa is constantly exposed to the microbiota and metabolites, which mediate the stimulation of the immune response to produce inflammation (Louis et al., 2014). Clinical research has shown that chronic inflammation increases the risk of neoplastic transformation (Greer & O’Keefe, 2011; Louis et al., 2014). IBDs, including Crohn’s Disease and colitis affect the host, increasing the risk for the development of CRC. The association between inflammation and cancer is well established; chronic inflammation leads to the production of several inflammatory cytokines and ROS, creating a micro-environment that promotes carcinogenesis (Louis et al., 2014).

Variations in the intestinal micro-environment that produce inflammation can promote alterations in the microbial community structure (Petersen & Round, 2014). Dysbiosis in the gastrointestinal microbiota promotes proliferation of bacterial populations that produce virulence factors such as cytotoxic necrotizing factor (Bacteroides fragilis) and colibactin (Escherichia coli) and the loss of protective populations (Louis et al., 2014). In this regard, cytolethal distending toxin and colibactin exert direct DNA damage through genomic instability (Schwabe & Jobin, 2013). Bacteroides fragilis and Streptococcus bovis are associated with CRC due to their ability to activate immune cells to release cytokines, such as IL-6 and IL-17 (Wu et al., 2009). Fusobacterium nucleatum adhesin has been reported to bind to E-cadherin and to activate β-catenin promoting the growth of tumour cells (Louis et al., 2014; Schwabe & Jobin, 2013), and to selectively recruit intestinal myeloid tumour cells to promote a pro-inflammatory micro-environment (Hooper & Macpberson, 2010).

Recent studies of colon adenoma in rat models have demonstrated that the microbiota induces IL-23, IL-17, IL-22 and IL-6 signalling as a result of defects in the integrity of the barrier and that antibiotic treatment abrogates tumorigenesis. In CRC models, the inhibition of IL-22 and IL-6 is relevant to the reduction of inflammation and tumour burden (Elinav et al., 2013; Francescone et al., 2014).

Recognized cause and effect: microbiota diagnostic and therapeutic tools

Although more conclusive studies and data linking microbiota to cancer are needed, the mechanisms by which the microbiota can modulate carcinogenesis provide alternative targets for cancer prevention strategies. Integration of information on microbial and mammal components of the human ‘super organism’ through metagenomics and metabolomics has enabled both the refinement and expansion of the existing catalogue of species associated with health and disease in various bodily organs and the development of new diagnostic and therapeutic strategies (Bultman, 2014; Dietert & Dietert, 2015; Schwabe & Jobin, 2013).

Diagnostic indicators

The validation and use of variations in the microbiota as an indicator of cancer (Fig. 1) can overcome the limitations of inter-individual reproducibility when considering the variations in the physiology, pathobiology, environment and lifestyle choices of the host (Khan et al., 2012). However, the common approaches to studying the microbial communities have been through metagenomics (Bultman, 2014; Khan et al., 2012), which does not evaluate gene expression, and hence does not determine the role of the microbiome. Further metatranscriptomic and metabolomic studies are needed to explore the metabolic activity of the microbiome. Large-scale investigations, clinical trials, a large cohort of patients and validation through in vivo and in vitro models are required to understand the microbiome composition, variability and function in tumorigenesis (Bultman, 2014; Fritz et al., 2013; Khan et al., 2012; Schwabe & Jobin, 2013). Other limitations to consider include the standardization of both sample stability and methods for comparative data analysis between research centres (Hebert et al., 2015).

Although the knowledge of the microbiota–cancer relationship is still limited, with findings primarily on CRC, future studies on the alterations of the microbiota could reveal...
new tumour markers or predictors (Gagnière et al., 2016; Sears & Garrett, 2014) that can be incorporated into epidemiological studies of cancer (Hebert et al., 2015).

**Alternative therapeutic**

Knowledge of the pathways mediated by microorganisms of the microbiota in the development of carcinogenesis suggests constitute possible approaches for prevention (Hassan et al., 2013; Sheflin et al., 2014) (Fig. 2):

- Probiotics, prebiotics or microbiota transplants can restore the equilibrium in the microbial community, and reduce pro-inflammation and toxic pathways that trigger carcinogenesis (Bultman, 2014; Cesaro et al., 2011; Clemente et al., 2012; Hassan et al., 2013; Khan et al., 1826; Garrett, 2015; Petersen & Round, 2014; Plottel & Blaser, 2011; Schwabe & Jobin, 2013).
- Genetically modified microbiota for the expression and silencing of specific enzymes will increase tumour suppressor substances or inhibit the development of tumour promoter species (Garrett, 2015; Hassan et al., 2013; Plottel & Blaser, 2011; Schwabe & Jobin, 2013; Sheflin et al., 2014). Approaches such as phages engineered using the CRISPR-Cas system and quorum systems may be used to posit alternatives for the control of dysbiosis (Belizário & Napolitano, 2015).
- Manipulation of the microbiota through antimicrobials: given the undesirable effects of broad spectra antibiotics, including the emergence of resistant strains and the harmful effects on normal microbiota, alternatives of narrow spectra such as bacteriocins should be considered (Walsh et al., 2014).
- With the impact of diet on the microbiota being known, diet can be modulated to avoid the proliferation of microorganisms that generate metabolic products deleterious for the host (Sheflin et al., 2014; Walsh et al., 2014).

Fig. 1. Cause and effect relationship. Molecular tools facilitate the study of prevalent microbial communities in groups of patients and healthy individuals to determine their role in the induction of carcinogenesis in different organs of the human body. Large-scale trials involving the study of different variables are needed, including metabolomic, metagenomic and metatranscriptomic analysis, as well as in vivo and in vitro investigations, to validate relationship hypotheses. These analyses have the potential to be used as diagnostic tools.
Use of pro-inflammatory genotoxins and enzymes involved with metabolite promoters of carcinogenesis as drug targets (Schwabe & Jobin, 2013).

The development of microbiota-based therapeutic approaches may be highly amenable for life-long use to prevent recurrence in patients with advanced tumours once remission occurs (Hassan et al., 2013).

During the last decades there has been an increasing interest in the effect of microbiota on the toxicity and efficiency of chemotherapy (Garrett, 2015). The function of microbiota on drug metabolism affects susceptibility to chemotherapy (Plottel & Blaser, 2011). The effect of oral bacteria β-glucuronidase on irinotecan (Schwabe & Jobin, 2013), mitigating its toxicity to the host, and the triggering effect of the microbiota on the intratumoural oxidative stress generated by oxaliplatin (Garrett, 2015; Iida et al., 2013; Viaud et al., 2013) have been studied. Studies have shown that dysbiosis has an impact on chemotherapies (Iida et al., 2013; Zambriniss et al., 2014). In immunostimulatory oligonucleotide therapies, the microbiota modulates the response to the release of proinflammatory factors (Perez-Chanona & Trinchieri, 2016).

**Conclusion**

Experimental and clinical evidence linking microbiota with different types of cancer is promising. Technological tools available today have provided an avenue to pursue investigations of causal hypotheses. International projects, such as HMP (Human Microbiome Project – USA), MetaHIT (Metagenomics of the Human Intestinal Tract) and IHMC (International Human Microbiome Consortium), have disclosed the relationship between microbiota metabolism and its implications for human health through characterization of composition and functional properties of microbial communities in different parts of the human body (Belizário & Napolitano, 2015; Cho & Blaser, 2012). Nevertheless, currently available methodologies have limitations, and studies
are needed to incorporate a larger number of patients and controls, as well as metagenomic, metatranscriptomic and metabolomic analyses, to integrate the structure of the community and its genetic content, expression and metabolism (Turnbaugh et al., 2007), and to expand knowledge of other areas of the human body that have a significant microbial load, such as skin, oral cavity and the female genital tract, but are as yet unexplored. Clearly, this is a nascent field of research worthy of continued investigation. Careful refinement of the methodological uses and the expansion of the number of related studies will promote understanding of the various effects of the bacterial microbiota in carcinogenesis and will provide new options for diagnosis and treatment. To advance knowledge of chronic human diseases, it is necessary to recognize that the human being is a holobiont. As mentioned by Blaser & Falkow (2009): ‘A greater understanding of the characteristics of a host’s genome and microbiota, and their interactions, will lead to individualized approaches to the prevention and treatment of specific diseases. We are at a scientific frontier’.

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