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THE MICROBIOME, IMMUNE DEVELOPMENT, CHRONIC INFLAMMATION, AND CANCER

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INTRODUCTION

Our microbiota is an integral part of our mammalian selves. Indeed, all multi-cellular eukaryotic hosts across the tree of life have an essential and characteristic microbiota that influences host development and resistance to disease. Within complex organisms such as vertebrates, we harbour numerous microbial communities whose composition and function are relevant to their habitat at different body sites, such as the intestines ("gut"), skin, and oral cavity. Our gut microbiota is perhaps the best studied, most abundant, and arguably, the most influential microbiota that impacts host phenotypes. In the recent decades, the development of several scientific tools has exponentially increased our understanding of the microbiota and interactions with its human host. These include model organisms, most notably "gnotobiotic" laboratory mice that are born and raised germ-free (GF) and then colonized with individual strains or groups of microbes. Through the use of GF and gnotobiotic mice, we have been able to demonstrate causality of specific microbes and microbial groups with distinct processes of immune development and non-infectious diseases like chronic inflammation and cancer, among others. To validate the physiologic relevance of observations made in model organisms with human disease, we can now survey the human microbiota at an unprecedented depth using culture-independent molecular methods (i.e. targeted 16S "microbiome" sequencing, metagenomics, metatranscriptomics, and metabolomics) coupled with sophisticated bioinformatics pipelines. An important finding from population studies of the microbiome has revealed that the compositional fluctuations in an individual's microbiome over time are less substantial than inter-individual differences at a particular stage in development. However, the developmental changes that occur during early life and over an individual's lifespan certainly shape the composition and function of the microbiota. Indeed, the composition and functional capabilities of the microbiota shape host development. The focus of the 2018 Old Herborn University Seminar Series 32 is "Ageing and the Microbiome". Accordingly, in this chapter we discuss the current state of knowledge regarding the influence of our mammalian microbiota on the immune system, chronic inflammation, and a prominent disease of the ageing – cancer.

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THE IMMUNE SYSTEM AND MICROBIOME IN AGEING

The immune system is our major host defence system that is educated early in life to distinguish harmful stimuli, including bacteria. It is broadly subdivided into two branches, the innate and adaptive immune systems. The innate immune response is the first line of defence towards invading pathogens. For example, this branch of the immune system is involved when host cell pattern recognition receptors are triggered by conserved pathogen specific molecules which promote an immediate and broad-spectrum protective response to pathogens. Conversely, the adaptive immune response relies on antigen presenting cells (APCs) to present bacterial products to effector T and B lymphocytes and create a pathogen-specific immunological memory and response. Through precise coordination, these systems provide host defence against foreign invaders. However, to efficiently mediate its response, the immune system must accurately distinguish between normal host-associated organisms and those that are potentially deleterious (*Chaplin*, 2010). Over the course of our lifetime, our immune system encounters a diverse range of stimuli in a variety of contexts, which challenges the ability of the immune system to differentiate between self and non-self (*Ponnappan* and *Ponnappan*, 2011). Alterations in this response can result in the development of a variety of diseases that include inflammatory bowel disease (IBD) and cancer (*Narendra* et al., 2013; *Gálvez*, 2014). As these immune responses are shaped over a lifetime, it indicates that age impacts the recognition of stimuli, which could transfer towards inappropriately reacting to residential host microbes. Inappropriate reactions to normal commensal bacteria may underlie a variety of pathogenic processes.

Microbial influences on immune development

Establishment of the microbiota begins from birth and continues until post-weaning (*Bergström* et al., 2014). During establishment, the microbiota is highly diverse and prone to fluctuations based on environmental and dietary changes (*Penders* et al., 2006). After 2-3 years of age, this complex community stabilizes with the majority of bacterial community members remaining unchanged throughout the lifespan of an individual (*Rajilić-Stojanović* et al., 2013). After stabilization, the core human microbiota mainly comprises the following phyla: *Bacteroidetes* and *Firmicutes*, with a smaller abundance of *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* (*Arumugam* et al., 2011). The ageing process strongly impacts the composition of the microbiota as individuals with increased frailty, an assessment of biological age based on current health status and life expectancy, lose bacterial diversity and form a *Bacteroidetes* dominant population (*Claesson* et al., 2011). While not directly correlated to chronological ageing, this loss of diversity most often begins between 75-80 years of age and is a form of dysbiosis that potentiates disease development (*Biagi* et al., 2010). These results are a generality from a diverse population, and only consider numerical age (lifespan) rather than the individual's ageing-related health status (health span). Despite this correlation, it is important to remember that the microbiota play a largely protective role in disease development by initiating and educating the host immune system (*Pickard* et al., 2017).

Early observations in GF mice demonstrated that the host microbiota is essential for the maturation of the immune system (*Clavel* et al., 2017; *Pickard* et al., 2017). In the absence of

a microbiota, GF mice have several immunological defects, including reduced lymphoid cell numbers and function (*Fiebiger* et al., 2016). For example, GF mice have less T helper type 1 (Th1) cells compared to their conventionalized counterparts (*Wu* and *Wu*, 2012). Th1 cells promote cell-mediated immune responses and phagocyte-dependent inflammation to target intracellular pathogens (*Damsker* et al., 2010). Th1 responses in GF mice can be restored through host colonization with a variety of microbes, including *Listeria monocytogenes* which promotes Th1 development through macrophage production of the T-cell-stimulating factor, interleukin 12 (IL-12) (*Hsieh* et al., 1993). In the gut, Th1 responses are driven by intracellular bacteria like *L. monocytogenes* (*Atarashi* et al., 2015). Additionally, GF mice have a reduced number of T helper type 17 (Th17) cells. Th17 cells are like Th1 cells in that they are pro-inflammatory; however, they drive the production of IL-17, a cytokine which mediates defence against extracellular pathogens and auto-immune disease (*Damsker* et al., 2010; *Wu* and *Wu*, 2012). Colonization of GF mice with intestinally adherent pathogens, such as Clostridia-related segmented filamentous bacteria (SFB), induces the development of Th17 cells in the small intestine by driving the release of serum amyloid A from intestinal epithelial cells (IECs). The release of serum amyloid A results in the production of innate lymphoid cell group 3 activating cytokines which upregulate the Th17 response (*Ivanov* et al., 2009). Fine-tuning of Th1 and Th17 responses is essential for immune tolerance towards the host microbiota, as seen in the case of IBD where aberrant populations of Th1 and Th17 cells lead to enhanced pathology (*Gálvez*, 2014). Therefore, underdevelopment of these responses may underlie the pathogenesis of other diseases associated with chronic inflammation, such as cancer (*Bailey* et al., 2014; *Vinay* et al., 2015).

The absence of a microbiota impacts most, if not all, aspects of the immune system (*Round* and *Mazmanian*, 2009). However, it is not fully understood where the window of opportunity lies for correcting many of these immune deficiencies. One study examining colonic invariant natural killer T (iNKT) cell populations revealed that this opportunity for modulation likely occurs in early life (*An* et al., 2014). At birth, GF mice have an enriched population of colonic lamina propria iNKT cells compared to specific-pathogen-free (SPF) mice (*Olszak* et al., 2012). iNKT cells are pro-inflammatory and mediate cell tolerance to commensal microbes (*Chandra* and *Kronenberg*, 2015). Colonization of adult (>5 weeks of age) GF mice with a complex microbiota does not influence the number and activity of iNKT cells (*An* et al., 2014). However, if the colonization occurs when GF mice are neonates, the number of iNKT cells is reduced and their later activation is well-controlled (*An* et al., 2014). This early education of the colonic iNKT cell population is important for limiting morbidity associated with IBD (*An* et al., 2014). This supports the idea that exposure to specific microbes and microbial products is needed within a certain developmental time for the host to appropriately educate their target immune population and prevent disease.

The gastrointestinal tract houses one of the largest and most diverse collection of microbes in the human body (*Thursby* and *Juge*, 2017). In the intestine, a single layer of epithelial cells separates the underlying mucosal immune system from the microbiota. Due to this proximity, much work has focused upon the protective role of the microbiota on inflammatory immune

responses that can lead to intestinal inflammation or colitis. For example, GF mice colonized with a beneficial non-toxin-producing strain of *Bacteroides fragilis* exhibit a marked expansion of regulatory T-cells (Tregs) in the intestinal mucosa (*Round* and *Mazmanian*, 2010). This expansion of Tregs limits inappropriate inflammation and is important for preventing autoimmune disease through immunotolerance towards self-antigens (*Round* and *Mazmanian*, 2010; *Rothstein* and *Camirand*, 2015). This protection is mediated by *Bacteroides spp.* through the production of polysaccharide-A (PSA), a commensal microbial surface molecule presented by APCs to modulate Treg specific immune responses. Indeed, colonization with PSA + *B. fragilis* protects mice from chemically TNBS-induced and *Helicobacter hepaticus*-induced colitis (*Round* and *Mazmanian*, 2010). Furthermore, colonization of conventional mice with *Clostridium butyricum* protects against experimentally-induced colitis by promoting the expansion of immunosuppressive interleukin-10 (IL-10) secreting macrophages and mature Tregs (*Hayashi* et al., 2013). *B. fragilis* and *C. butyricum* belong to the most highly represented bacterial phyla within the murine and human microbiota (*Bacteroidetes* and *Firmicutes*, respectively) and mechanistically represent some of the many ways the commensal microbiota can modulate host immune responses to prevent the onset of disease (*Belkaid* and *Harrison*, 2017; *Clavel* et al., 2017; *Pickard* et al., 2017).

The immune system and ageing

The presence of a microbiota in early life is essential for immune system maturation. However, education of the immune response is a lifelong process. Alterations to the innate and adaptive immune systems which occur with increased frailty are linked to a complex biological process known as immunosenescence (*Fülöp* et al., 2016). Specific changes associated with immunosenescence can best be understood through functional differences within the unique cell types of the innate and adaptive immune systems. For cells of the innate immune system, there are reported functional differences for every major cell type (*Castelo-Branco* and *Soveral*, 2014). However, the most distinct differences are within neutrophil and macrophage populations. Neutrophils isolated from the blood of individuals, aged 62-83, displayed reduced phagocytic capabilities and decreased production of reactive oxygen species (ROS) when infected with *Staphylococcus aureus* (*Wenisch* et al., 2000). These neutrophils, versus cells isolated from younger adult patients, also had impaired bactericidal activity (*Wenisch* et al., 2000). Neutrophils are the first line of defence towards invading pathogens. Therefore, immunosenescence related changes to this cell type suggest an age-related decline in pathogen tolerance (*Chaplin*, 2010). Similarly, primary macrophages isolated from aged mice (18-24 months old) have impaired phagocytosis and reduced ROS production in response to infection when compared to macrophages isolated from young mice (2-3 months old) (*Davila* et al., 1990; *Swift* et al., 2001). Additionally, macrophages from aged mice display altered antigen presentation and reduced production of pro-inflammatory cytokines (*Herrero* et al., 2001; *Renshaw* et al., 2002). Alterations in macrophage antigen presentation and cytokine release may lead to altered immune signalling between the innate and adaptive immune systems, resulting in a weakened immune response (*Chaplin*, 2010). Overall, age-related changes to the innate immune system strongly reduce the host's initial re-

sponse to pathogens.

While changes in the innate immune system have been noted with ageing, alterations to the adaptive immune system may be more pronounced (*Linton* and *Dorshkind*, 2004; *Castelo-Branco* and *Soveral*, 2014). The adaptive immune system is used for long-term protection from environmental insult and invading pathogens. Therefore, long-term education of this subsystem could have additive effects on immunosenescence. B-cells are one of the major cell types of the adaptive immune system. The population of B-cells is divided into plasma cells or memory B-cells. Plasma cells produce pathogen specific antibodies, while memory B-cells provide long-term recognition of antigens (*Eibel* et al., 2014). Peripheral blood isolated from elderly individuals (aged 86-94 years) showed a reduction in B-cell population diversity, likely due to a decrease in memory B-cells (*Gibson* et al., 2009). This decline in B-cell diversity was linked to increased frailty and may be used as a predictor for general health status (*Gibson* et al., 2009). A reduction in memory B-cells may cause an inappropriate immune response towards the microbiota, as B-cells are important for establishing the distinction between pathogenic and commensal bacteria (*Eibel* et al., 2014).

T-cells are the second major cell type of the adaptive immune system. Under normal immunological conditions, T-cells differentiate into either T Helper (Th) cells or natural killer T (NKT) cells. This differentiation is based on antigen presentation by APCs and is necessary to form a pathogen-specific immune response (*Chandra* and *Kronenberg*, 2015). Populations of T-cells isolated from the peripheral blood of elderly humans (aged 72-89 years) showed a reduction in the proportion of NKT-cells versus cells isolated from young patients (aged 25-30 years) (*DelaRosa* et al., 2002). This finding was matched by a study which showed a reduction in the proportion of NKT-cells isolated from the peripheral blood of adults aged 61 years and over (*Jing* et al., 2007). Additionally, NKT-cells isolated from the liver of aged mice (aged >20 months) demonstrated a decline in cytotoxic function, and reduced cytokine release versus T-cells isolated from young mice (aged 2 months) (*Mocchegiani* et al., 2004). Under non-immunosenescence conditions, NKT-cells respond to antigenic activation by robust proliferation and directed cytotoxicity (*Chandra* and *Kronenberg*, 2015). Therefore, the reduced proportion of NKT-cells and cytotoxic ability in elderly organisms may exacerbate disease development by weakening the host's response to pathogens.

The host microbiota initiates immune system maturation in early life. However, to keep up with a lifelong antigenic load, the immune response must be fine-tuned and properly educated across the lifespan. Changes to the immune system that occur with age are noted within immunosenescence whereby functional differences develop within innate and adaptive immune cell populations. Despite these observations, it remains unclear how these immunological changes impact cellular crosstalk and overall immunocompetence. On top of this, how the microbiota impacts the immune system during immunosenescence remains to be elucidated. It is likely that changes to the immune system result in an inappropriate response towards commensal microbes, as indicated by diseases like IBD (*Sun* et al., 2015). Therefore, these inappropriate reactions to the native microbiota may contribute to the development of chronic inflammation and the onset of age-related diseases, such as cancer (*Tilg* et al., 2018).

MICROBIOME AND CANCER – A DISEASE OF AGEING

Cancer is considered a disease of old age. As life expectancy increases, the estimated rate of cancer is predicted to increase by 45% from 2011 to 2030 in the United States (*Smith* et al., 2009). It is also estimated that by 2030, individuals 65 years and older will contribute to 70% of all cancers in the United States (*White* et al., 2014). The risk of developing cancer increases dramatically with age as the duration of time in which an individual is exposed to carcinogens increases (*Harding* et al., 2012), the proliferative capacity of ageing cells decreases (*Pompei* et al., 2001), and immunological competence decreases (*Bonafè* et al., 2002). Cancer typically results from a series of genetic mutations or epigenetic modifications that develop sequentially overtime (*Loeb* et al., 2003). The colon, which harbours the largest and most diverse microbiota of all organs, has the highest incidence rate of all reported cancers in the 85+ population (*Thakkar* et al., 2014). Over the past decade, we have become increasingly aware of the roles that the microbiota play in the development of cancer and modulation of cancer therapies. We have also elucidated several mechanisms underlying the microbial influences on cancer. However, these roles are diverse and seem to influence many aspects of immune and cancer development (*Fulbright* et al., 2017). Microbes can contribute to the onset and progression of cancer through direct means, such as by producing genotoxins, and indirect means through the modulation of immune responses to tumours and immunotherapy (*Rhee* et al., 2009; *Arthur* et al., 2012; *Kostic* et al., 2013; *Boleij* et al., 2015; *Gopalakrishnan* et al., 2018). Additionally, several members of the native microbiota can alter chemotherapeutic drugs, resulting in unpleasant side effects for the host or even rendering them clinically inert (*Al-Dasooqi* et al., 2011; *Geller* et al., 2017). It is therefore important to divulge the significance of microbial interactions on age-related diseases, such as cancer, in order to fully understand disease progression and design suitable therapies.

The ability to manipulate the microbiota using GF and gnotobiotic mice has demonstrated the importance of the microbiota on immune system development. The microbiota assist in training the immune system to recognize harmful and non-harmful stimuli, both from the environment and within the self. Here we will discuss known mechanisms by which the microbiota can influence anti-cancer chemotherapeutic activities and anti-tumour immunomodulatory responses. For example, *Clostridia spp.* are capable of suppressing the body's anti-tumour immune responses. Host-derived primary bile acids are converted into secondary bile acids by the gut microbiota, primarily members of the genus *Clostridia,* and circulated systemically throughout the body via hepatic circulation (Ridlon et al., 2006). Previous work illustrated that secondary bile acids can increase the risk of obesity-associated hepatocellular carcinoma in susceptible mice (*Yoshimoto* et al., 2013). Recent data suggests that antibiotic elimination of the gut microbiota in mice decreases both primary and metastatic tumours within the liver by facilitating the build-up of primary bile acids, which trigger liver-specific NKT-cell recruitment to target cancer cells (*Ma* et al., 2018). The profound effects bacteria illicit on cytotoxic immune cells provide key insights on how the native microbiota influence host anti-tumour responses.

Another example, *Fusobacterium nucleatum*, a Gram-negative oral commensal overrepresented in colorectal carcinoma, can promote tumourigenesis through the modulation of the innate immune system (*Castellarin* et al., 2012; *Kostic* et al., 2013). A known target is the natural killer (NK) cell, which kills compromised host cells, such as infected or cancerous cells. *F. nucleatum* inhibits the cytotoxicity of NK-cells via the Fusobacterium protein Fap2 which binds the NK-cell inhibitor receptor TIGIT (T-cell immunoglobulin and ITIM or immunoreceptor tyrosine-based inhibition motif domain) (*Gur* et al., 2015). In addition to targeting the immune system, it should be mentioned that *F. nucleatum* exerts pro-carcinogenic activities directly on epithelial cells through β-catenin signalling, altering cell fate (*Rubinstein* et al., 2013). *F. nucleatum* can also alter the efficacy of chemotherapeutic drugs by inhibiting host cell apoptotic pathways (*Yu* et al., 2017). *F. nucleatum* is a prime example of one species of the microbiota that exhibits a variety of different effects on the host to mediate tumourigenesis and hinder cancer therapy. In the next few sections, we will discuss a variety of known bacterial mechanisms that act upon cancer development and treatment.

Cancer immunotherapy and the microbiota

Several independent groups have recently demonstrated that some members of the microbiota play critical roles in determining patient responsiveness to cancer immunotherapy. The exact mechanisms by which individual species of bacteria exhibit these effects are not fully understood. However, current data suggest that bacterial modulation of the immune system may be one critical mode of altering host response to cancer therapy. Recent data regarding anti-PD1 therapy supports this notion. Anti-PD1 treatment is a type of immune checkpoint inhibitor that enhances anti-tumour immune responses by maintaining T-cell activation via blocking the immune inhibitory receptors programmed death ligand-1 and 2 (PDL-1 and PDL-2) (*Shields* et al., 2017). Anti-PD1 therapy is often prescribed to patients with lung cancer and advanced melanoma. However, the efficacy of anti-PD1 immunotherapy ranges from only 19 to 43% for both cancer types (*Jiang* et al., 2015; *Larkin* et al., 2015). Several members of the microbiota are enriched in PD-1 responders, including *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium* (*Matson* et al., 2018). *Faecalibacterium,* an abundant Gram-positive genus of commensals in the human gut (*Shabbir* et al., 2016), was also enriched in PD1-responders (*Gopalakrishnan* et al., 2018). Tumour-bearing mice that were given faecal microbiota transplants (FMTs) from PD1-responders exhibited decreased tumour burden and tumour size when receiving anti-PD1 therapy. *Faecalibacterium* promoted cytotoxic (CD8+) T-cell recruitment to tumours, which may be an important mechanism underlying the ability of this bacterial group to enhance anti-PD1 responses and reduce tumour burden (*Gopalakrishnan* et al., 2018). Similarly, another group demonstrated that FMTs from PD-1 responders enhanced PD-1 treatment in recipient mice, which was further augmented with oral supplementation of the commensal *Akkermansia muciniphila* (*Routy* et al., 2018). Antibiotic treatment reduced the efficacy of PD-1 immunotherapy in mice, consistent with clinical reports of reduced PD-1 efficacy in patients simultaneously taking antibiotics (*Routy* et al., 2018). These studies demonstrate that multiple species of bacteria have the capability

of altering immunotherapeutic responses in patients. Moving forward, it may be critical to consider the contributions of these microbial communities when developing anti-tumour immunotherapies.

Chemotherapy and the microbiota

Multiple members of the microbiota can differentially influence cancer chemotherapy, with some enhancing and some inhibiting the clinical effects of chemotherapeutic drugs. An important early observation was that genotoxic platinum chemotherapies, including oxaliplatin and cisplatin, were ineffective in tumour-bearing GF mice (*Iida* et al., 2013). Thus, the presence of a complex microbiota is essential for these chemotherapies (*Iida* et al., 2013). Platinum chemotherapies utilize ROS to induce cytotoxicity. The DNA damage induced by cisplatin is augmented via the production of mitochondrial ROS within cancer cells themselves (*Marullo* et al., 2013). Data also indicates that the ROS production from tumour-associated inflammatory cells are critical for the efficacy of these platinum chemotherapeutic drugs (*Iida* et al., 2013). It may be that in the absence of a native microbiota, inflammatory cells are not effectively primed to produce ROS during development, leading to shortcomings in ROS production later in life. This data highlights the influential capacity of the microbiota on the host and how in their absence the immune system may have substantial deficits in anti-tumour responses. Conversely, the microbiota can have negative effects on chemotherapeutic efficacy. Deep sequencing for microbes within pancreatic tumour biopsies revealed that 57.5% of pancreatic tumour tissues tested (65 of 113 samples) were positive for bacterial reads, with *Gammaproteobacteria* being the most abundant (51.7% of reads) (*Geller* et al., 2017). Interestingly, 98.4% of *Gammaproteobacteria* contain genes that encode a specific isoform of the enzyme cytidine deaminase (CDD_L), which has the ability to breakdown gemcitabine and confer chemotherapeutic resistance in tumour tissues (*Geller* et al., 2017). Accordingly, bacterial migration from the gastrointestinal tract into the pancreatic ducts and tumour tissue may be a significant source of drug failure in clinical pancreatic cancer cases. Gut bacteria are also responsible for re-activating chemotherapeutic drugs in the distal intestine. Irinotecan, a chemotherapeutic drug used to treat colorectal cancer (CRC), is inactivated by the liver, but reactivated into the activate drug by *Clostridia spp.* through β-glucuronidases in the gut (*Stringer* et al., 2009). This re-activation contributes to the typical unpleasant gastrointestinal side effects of irinotecan therapy, including mucositis and diarrhoea (*Stringer* et al., 2009; *Al-Dasooqi* et al., 2011). This evidence illustrates the profound impact the native microbiota can have on the response to cancer therapies. Therefore, future treatment plans should account for the influence of these patient-specific microbial factors to ensure successful outcomes.

Direct effects of the microbiota on tumourigenesis

Specific members of the microbiota have the capacity to directly contribute to tumourigenesis (*Schwabe* and *Jobin*, 2013; *Fulbright* et al., 2017). Commensal *Enterobacteriaceae*, including several strains of *Escherichia coli,* are capable of inducing DNA damage in mammalian cells by producing a genotoxin termed colibactin (*Cuevas-Ramos* et al., 2010; *Arthur* et al., 2012). The bacterial polyketide synthase (*pks*) pathogenicity island encoding colibactin is up-regulated in CRC models and

the presence of these gene products promotes tumourigenesis by inducing double-stranded DNA breaks (*Nougayrede* et al., 2006; *Arthur* et al., 2014; *Tomkovich* et al., 2017). Colibactin can also induce premature cellular senescence in cells that initially survive the DNA damage (*Secher* et al., 2013; *Cougnoux* et al., 2014) Furthermore, the *pks* pathogenicity island is overrepresented in the microbiota of CRC and IBD patients (*Arthur* et al., 2012; *Buc* et al., 2013; *Prorok-Hamon* et al., 2014). This suggests that bacterial genotoxins likely contribute substantially in the development of human cancer and chronic inflammatory diseases.

Bacteria also have the capacity to induce a pro-tumourigenic environment through chronic inflammation. Enterotoxigenic *B. fragilis,* a member of the most abundantly represented genus in the gut, produces its own flavour of toxin called *B. fragilis*-derived toxin (BFT) (*Wu* et al., 2002). BFT is a zinc-dependent metalloprotease that can induce colitis and promote tumourigenesis through the generation of ROS and subsequent initiation of DNA damage in epithelial cells (*Goodwin* et al., 2011). Enterotoxigenic *B. fragilis* robustly activates Th17 immune responses, which involves the inflammatory cytokine interleukin-17 (IL-17), and may lower host anti-tumour immune responses, encouraging unhindered tumour growth (*Wu* et al., 2009; *Geis* et al., 2015). Enterotoxigenic *B. fragilis* is overrepresented in patients with CRC when compared to healthy individuals (*Boleij* et al., 2015) and exacerbates tumourigenesis in susceptible mice (*Wu* et al. 2009). Interestingly, the tumourigenic effects of *pks+ E. coli* and enterotoxigenic *B. fragilis* act synergistically *in vivo* to quicken tumour onset and increase mortality in susceptible mice beyond the capability that either species has individually (*Dejea* et al., 2018). Given that the native microbial community is quite complex, the cumulative effects of microbial products on the host may significantly contribute to the onset of cancer.

One of the earliest identified microbial suspects of inflammation-mediated cancer development is *Helicobacter pylori*. While widely considered a pathogen, *H. pylori* is estimated to be present in the gastrointestinal tract of over half of the human population worldwide and a major risk factor for gastric adenocarcinoma (*Arthur* and *Jobin*, 2011; *Hooi* et al., 2017). It is estimated that *H. pylori* infection increases the attributable risk of gastric cancer by 73% (*Herrera* and *Parsonnet*, 2009). Chronic *H. pylori* infection results in inflammation and tissue damage by the bacterial virulence factor CagA (cytotoxin-associated gene A), which initiates the development of the hallmark precursory lesions of gastric cancer, including intestinal metaplasia and dysplasia (*Díaz* et al., 2018). It remains unclear why *H. pylori* infection only progresses to malignancy in a subset of infected individuals; however, it is postulated that host immune responses and the genetics of both host and bacteria contribute to neoplastic development (*Polk* and *Peek*, 2010). The evidence presented here illustrates the diverse microbial mechanisms that contribute to tumourigenesis, whether that be by directly targeting the DNA for damage through a toxin or by providing an augmented environment for unrestricted cellular proliferation.

In summary, the mechanisms by which the native microbiota influences cancer development and therapy are numerous and diverse. As more data surfaces, it will be imperative to synthesize and apply knowledge on microbial contributions towards cancer

development and treatment. By doing so, we can more effectively assess cancer risk and ultimately design more potent therapies.

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