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Review article

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Bacteria in cancer initiation, promotion and progression

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Abstract

Cancer cells originate from a series of acquired genetic mutations that can drive their uncontrolled cell proliferation and immune evasion. Environmental factors, including the microorganisms that colonize the human body, can shift the metabolism, growth pattern and function of neoplastic cells and shape the tumour microenvironment. Dysbiosis of the gut microbiome is now recognized as a hallmark of cancer by the scientific community. However, only a few microorganisms have been identified that directly initiate tumorigenesis or skew the immune system to generate a tumour-permissive milieu. Over the past two decades, research on the human microbiome and its functionalities within and across individuals has revealed microbiota-focused strategies for health and disease. Here, we review the evolving understanding of the mechanisms by which the microbiota acts in cancer initiation, promotion and progression. We explore the roles of bacteria in gastrointestinal tract malignancies and cancers of the lung, breast and prostate. Finally, we discuss the promises and limitations of targeting or harnessing bacteria in personalized cancer prevention, diagnostics and treatment.

Sections

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Introduction

Microorganisms are small but potent influencers of both immunity and cancer. Over the past two decades, the field of microbiome sciences has grown tremendously, driven by next-generation sequencing, microbiome-oriented computational pipelines and wet-laboratory technologies that enable hypothesis testing at high and low throughput (for example, transposon-based mutagenesis methods and gnotobiotics). In parallel and consequently, microbes have emerged as a key factor linking the immune response and cancer. Specific microbial communities can now be adequately quantified, correlated with specific disease status and mechanistically interrogated using preclinical models[1,2].

The gut microbiota can be disrupted by malnutrition, overnutrition, inflammatory and infectious diseases, especially those of the gastrointestinal tract, and through pharmaceuticals[3,4]. Repeat exposures to antibiotics over a lifetime and during postnatal development are established contributors to dysbiosis (an unhealthy shift in microbial community abundance, composition and function), and have been linked to certain types of cancer[5,6]. Although long-term use of antibiotics may increase the risk of developing breast cancer and even colonic adenomas, no causal relationship has yet been uncovered[5,6]. Further research is needed to fully elucidate these mechanisms and develop strategies to mitigate the cancer risk meted out by the microbiome associated with not only antibiotics but also proton pump inhibitors (PPIs), β-adrenergic receptor modulators and other medications[7]. Overall, more clinical investigations and preclinical research focused on elucidating the underlying mechanisms are needed to refine our understanding of the effects of dysbiosis on tumour initiation and progression inside and outside the gastrointestinal tract.

Approximately 20% of all cancers have been robustly linked with specific viral or microbial infections; however, these malignancies are driven by a handful of viruses, for example human papillomavirus (HPV subtypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 for cervical cancer, and HPV 16 for head and neck cancer squamous cell carcinomas), Epstein–Barr virus (for lymphoma), hepatitis B and C viruses (for hepatocellular carcinoma) and human T cell lymphotropic viruses (for leukaemia and lymphoma), and principally by one bacterium (*Helicobacter pylori* for gastric cancer)[8,9]. Bacteria are increasingly recognized as key players in the tumorigenesis of several types of cancer[10–18] and numerous studies also support a role for the gut microbiota in modulating responses to cancer immunotherapy and targeted therapies, heightening the theranostic opportunities for the microbiome[11,19–25]. Although leveraging the human microbiome for cancer research holds potential for early detection, prevention and treatment, it still has critical limitations. Recent findings have highlighted that the composition of the gut microbiome diverges greatly even among healthy individuals. These effects are driven by exposures to diseases, medications and dietary pattern[4,26–29]. Thus, it is necessary to consider differences among patient cohorts when analysing the landscape of the gut and intra-tumoural microbiome to identify the specific mechanisms implicated in cancer initiation, promotion, progression and response to therapy.

In this Review, we discuss recent studies that investigate microbiota-mediated carcinogenesis. We explore pro-oncogenic microbe-driven mechanisms at different body sites, with a focus on microbially induced mutagenesis (cancer initiation) or through sustained and chronic inflammation (cancer promotion), that influence the evolving tumour microenvironment (TME) towards metastasis (cancer progression). Here we have focused specifically on which microbial entities are able to directly interact with mammalian host cells, and/or produce oncometabolites that can trigger inflammation and host cell transformation[8]. We also briefly explore the potential and limitations of the microbiota in personalized cancer prevention, diagnostics and treatment. Although our Review centres on bacteria, there are emerging, potential roles for fungi and gut-resident viruses in tumorigenesis given their detection in tumour tissues[30–32].

Microbes of the internal organs

Of all the microbial niches of the human body, the gastrointestinal tract houses the highest number of microorganisms, and microbial densities are at their greatest within the colon[33]. Dysbiosis can alter intestinal homeostasis and correlates with gut-localized and systemic diseases[34]. Given the fascinating associations between the faecal microbiota and human diseases, it is not surprising that the microbiota is garnering substantial attention in cancer research. Over the past two decades, preclinical models have helped reveal the mechanisms by which several microorganisms, enriched in human tumour tissues, enhance tumorigenesis via their direct effects on epithelial cell neoplastic transformation[10,35] (Fig. 1). Below, we review these mechanisms in gastrointestinal organ cancers and in cancers of the lung.

Stomach

Helicobacter pylori. *H. pylori*, a gram-negative bacterium and well-recognized oncomicrobe, is categorized as a carcinogen by the World Health Organization (WHO) and contributes to more than two thirds of gastric cancer worldwide[10,36,37]. *H. pylori* is a genus and species taxonomic designation for a large number of bacterial strains that share a high degree of similarity as defined by DNA relatedness as well as specific phenotypic and biochemical features. In classical bacteriology, the term strain is used to refer to a specific isolate in pure monoculture. Taxonomically, the terms bacterial species and bacterial strain are distinct but, unfortunately, have been used with various intended meanings. Often, microbiologists refer to 'pathogenic' versus 'harmless' strains of *H. pylori* and apply the term to other bacteria as well, such as *Escherichia coli*, which have many environmental, human resident and pathogenic members or strains. Thus, for some bacteria, strains differ in some key phenotypic manner — for example, disease-causing in humans. Others use the term strain to denote within-species genetic variation. The imprecise usage of the word strain, the complexity of grouping bacteria and recent reclassifications, as well as the vast degree of within-species genomic variation was recently reviewed by Van Rossum et al.[38]. *H. pylori* has many strains based on genetic variation and phenotypic differences. Often the presence of a particular virulence gene called cytotoxin-associated gene A (*CagA*), discussed further below, is used to define *H. pylori* isolates, as gastric colonization with *CagA*-expressing isolates increases the risk of peptic ulcer disease and is correlated with increased risk for gastric adenocarcinoma[37,39].

Decades of research unravelling the connections between *H. pylori*, gastritis and peptic ulcer disease culminated in Barry Marshall and Robin Warren receiving the 2005 Nobel Prize in Physiology or Medicine[40]. *H. pylori* can infect the stomach, an organ once considered sterile due to its acidic pH[41]. To survive and replicate under those harsh conditions, *H. pylori* elevates the gastric pH by secreting urease, an enzyme that generates ammonia from urea[42]. PPIs, used to treat gastroesophageal reflux disease, gastritis and peptide ulcers, increase the intragastric pH, and when combined with antibiotic cocktails (for example, amoxicillin, clarithromycin and metronidazole) are highly efficient at clearing *H. pylori* infections, thus reducing gastric cancer incidence[43]. Conversely, both frequent use of antibiotics and

long-term use of PPIs can cause dysbiosis which has been correlated with an overall increased risk of gastric cancer by 2.4-fold (refs. 44–49).

Over the past several decades, many investigators have contributed to elucidating the key molecular pathways and specific proteins critical for *H. pylori* pathogenesis. Binding of *H. pylori* to host cells and tissues is the first step in its bacterial pathogenesis. *H. pylori* attaches to gastric epithelial cells via its adhesin HopQ and engages specific cellular carcinoembryonic antigen-related cell adhesion molecules (CEACAMs), essential for translocation of its virulence factor CagA into the cytoplasm of host cells via the type IV secretion system (T4SS)[50]. CagA increases cell proliferation: it binds to the cytoplasmic domain of E-cadherin, disrupting the formation of the E-cadherin–β-catenin complex, inducing β-catenin translocation to the nucleus leading to activation of the Wnt–β-catenin pathway, crucial for the self-renewal of cancer stem cells[37]. The tumorigenic potential of *H. pylori* is mediated by direct effects on gastric epithelial cells and by its induction of chronic inflammation, as it can activate the nuclear factor-κB (NF-κB) pro-inflammatory pathway via its lipopolysaccharide (LPS), peptidoglycan or CagA[51–54].

H. pylori infection is associated with not only gastric adenocarcinoma but also mucosa-associated lymphoid tissue (MALT) lymphoma, a rare subtype of non-Hodgkin lymphoma arising from B cells in the stomach. *H. pylori* is detected in more than 90% of MALT lymphoma cases[55] and CagA is a key driver in the pathophysiology[55]. After translocation into host cells, CagA can be phosphorylated by kinases from the Src family and bind SHP-2 in the cytoplasm. CagA–SHP-2 complexes stimulate cell proliferation and inhibit apoptosis via activation of the ERK–MAPK signalling pathway, further increasing the expression of Bcl-2 and Bcl-XL, two anti-apoptotic proteins[55,56]. Notably, gastric MALTs are often effectively treated with *H. pylori*-directed antibiotics rather than requiring traditional neoplastic therapy[57–59].

The connections between microbial infection and cancer are not always straightforward. Epidemiological studies have found that *H. pylori* infection may be associated with a reduced risk of oesophageal adenocarcinoma[60,61]. This observation raises many questions, such as whether microbial biogeography influences susceptibility to cancer, whether there is disease-causing heterogeneity of bacteria within a given species, how prior or current infection with an organism such as *H. pylori* can potentially reduce the risk for developing certain cancers[62] and whether a particular bacterial isolate can trigger host cell mutagenesis versus chronic inflammation.

Balancing the *H. pylori* gastric cancer risk versus the risks of screening and treatment and the potential benefits of carriage, eradication strategies are still under investigation, even in high-endemic areas where gastric infection can be asymptomatic. Rather than global eradication, as greater than 50% of the world's population may harbour *H. pylori*, risk within a particular location or community should be considered. Given that *H. pylori* infection can easily spread within family and households, as source control is challenging, efforts backed by data-driven risk assessments that incorporate practical and cost-effective screening methods remain an active area of clinical investigation[63,64].

Gallbladder

***Salmonella enterica*.** Gallbladder cancer (GBC) is the most common malignancy of the biliary tract, yet a relatively rare type of cancer overall. It is an aggressive cancer with high metastatic potential and striking geographic variation[65]. Both chronic bacterial and parasitic infections increase GBC risk. Specifically, there are long-standing associations between GBC and *Salmonella* infections[66]. Carriers and those chronically infected with typhoid *Salmonella* (*Salmonella typhi* and *Salmonella paratyphi*) are at high risk for GBC and its prevalence is therefore much higher in areas where typhoid is endemic (for example, northern India)[67].

S. typhi is a potent oncomicrobe for GBC[68]. Via its type 3 secretion system, *S. typhi* releases its virulence factor AvrA, which activates Wnt–β-catenin signalling and the janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway. The *S. typhi* typhoid toxin triggers DNA double-stranded breaks via its CdtB subunit, which possesses DNase-like activity[69–72]. In a study by Sepe et al., the researchers found that gallbladder organoids infected with *S. typhi* exhibit genomic instability, strengthening the direct evidence for its role in GBC initiation[16]. The Cdtb subunit can damage DNA without triggering cell-cycle arrest leading to transformation over time. However, the exact means by which *S. typhi* impairs cell-cycle arrest remain unclear. Investigators also uncovered a paracrine DNA damage effect in which non-infected bystander cells also exhibit genomic instability, ultimately leading to malignant transformation. This study highlights the relevance of an increasingly used model system (that is, organoids for characterizing the molecular changes induced by a specific bacterium) for studies of carcinogenesis and its importance for identifying deployable, precision medicine therapies. Overall, approaches aimed at GBC prevention, namely vaccination with boosters and screening in typhoid endemic areas with antibiotic sensitivity-guided treatment, may be impactful given the dearth of effective GBC treatments[73].

Colon

The microbiota concentration increases steadily throughout the gastrointestinal tract, reaching its highest density in the colon, which harbours about 10^{12} bacteria per gram within its lumen[74]. Given the high bacterial load of the colonic lumen, colon cancers have a relatively high microbial biomass compared with other mucosal and non-mucosal tumours[75]. Colorectal cancer (CRC) tumours harbour live microbes and enrichments of certain bacteria in CRC tissues correlate with worse clinical outcomes. Research supports that these oncomicrobes exhibit causative roles in mouse models of CRC and have identified how they may contribute to CRC progression and spread[10,15,76–81]. One such mechanism involves the release of genotoxins, molecules that can induce DNA damage and cancer-associated mutations within host cells[82,83]. Genotoxins represent one of numerous strategies that microorganisms have evolved to allow them to compete against other microbes in the human gastrointestinal tract. These microbial warfare techniques can involve targeting many cell processes and functions, and was recently comprehensively reviewed[84]. Some microbes can release antimicrobials that damage other microbes' DNA (for example, genotoxins) and coincidentally target host cells. Colibactin-producing *E. coli* (referred to as *pks*$^+$ *E. coli*) and enterotoxigenic *Bacteroides fragilis* (ETBF) are implicated in colonic tumorigenesis via production of toxins that have been studied for many decades whereas their implications for CRC are more recent. Additional organisms, some from the oral cavity, are garnering increased interest as well through non-toxin-mediated promotion of CRC.

In assessing how the microbiome may affect cancer risk, investigators should not only consider the presence or enrichment of potential disease-causing microbes but also the reduction or absence of microbes that may heighten resistance to carcinogenesis[85]. In a recent study, Zagato et al. found that *Faecalibaculum rodentium* and its human homologue, *Holdemanella biformis*, protect against the development

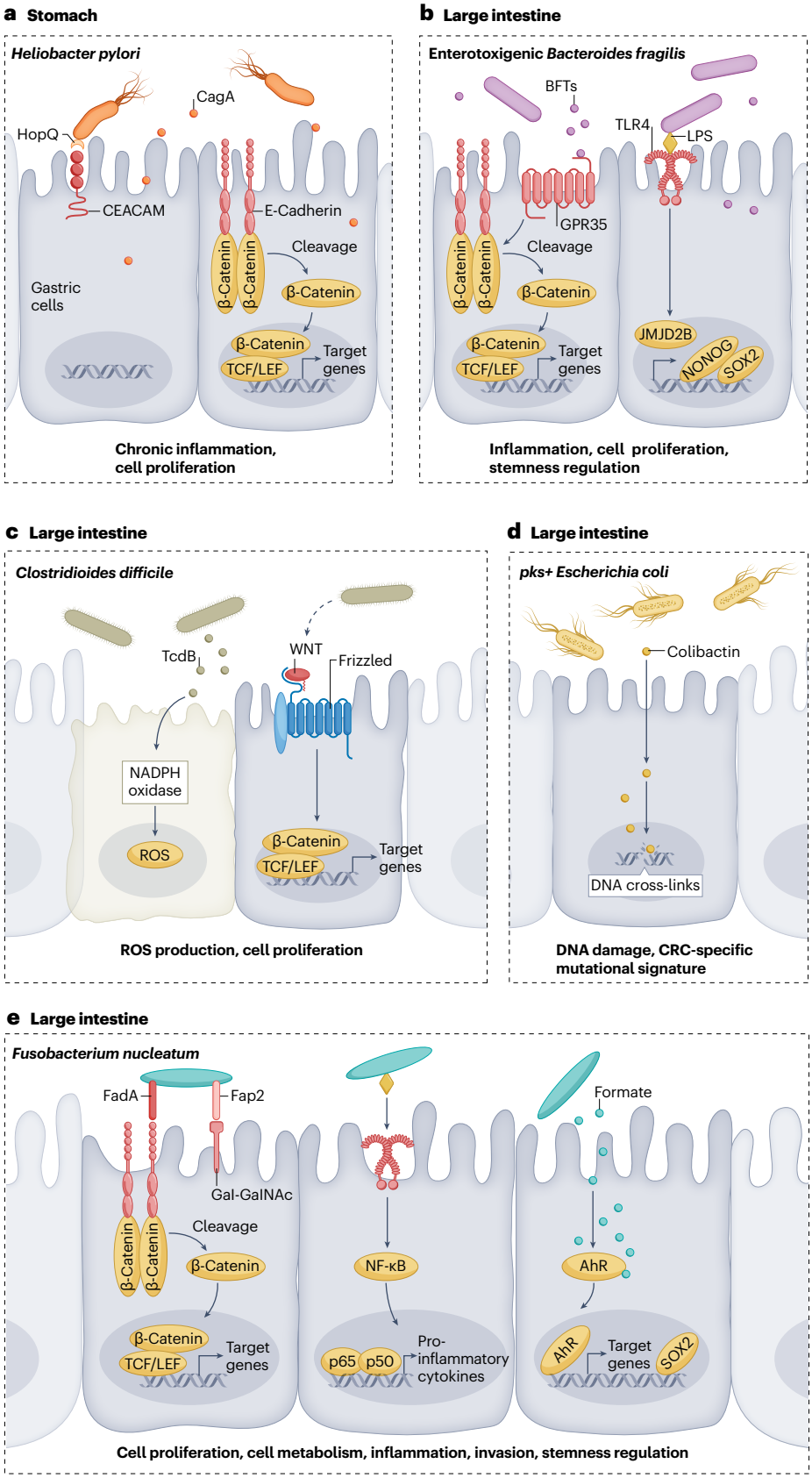
a Stomach
Heliobacter pylori
CagA
HopQ
CEACAM
E-Cadherin
Cleavage
β-Catenin
β-Catenin
β-Catenin
β-Catenin
TCF/LEF
Target genes
Gastric cells
Chronic inflammation, cell proliferation
b Large intestine
Enterotoxigenic Bacteroides fragilis
BFTs
TLR4
LPS
GPR35
Cleavage
β-Catenin
β-Catenin
β-Catenin
β-Catenin
TCF/LEF
Target genes
JMJD2B
NONOG
SOX2
Inflammation, cell proliferation, stemness regulation
c Large intestine
Clostridioides difficile
TcdB
WNT
Frizzled
NADPH oxidase
ROS
β-Catenin
TCF/LEF
Target genes
ROS production, cell proliferation
d Large intestine
pks+ Escherichia coli
Colibactin
DNA cross-links
DNA damage, CRC-specific mutational signature
e Large intestine
Fusobacterium nucleatum
FadA
Fap2
Gal-GalNAc
Cleavage
β-Catenin
β-Catenin
β-Catenin
β-Catenin
TCF/LEF
Target genes
NF-κB
p65
p50
Pro-inflammatory cytokines
Formate
AhR
AhR
Target genes
SOX2
Cell proliferation, cell metabolism, inflammation, invasion, stemness regulation

Fig. 1 | Mechanisms of bacteria-associated tumorigenesis in gastrointestinal organs. a, *Helicobacter pylori* binds to gastric epithelial cells via HopQ and engages specific cellular carcinoembryonic antigen-related cell adhesion molecules (CEACAM1, CEACAM3, CEACAM5, CEACAM6). Its virulence factor cytotoxin-associated gene A (CagA), produced by the *cag*-type IV secretion system (*cag*-T4SS), modulates the Wnt–β-catenin pathway, which regulates cell proliferation and apoptosis. Upon translocation to the nucleus, β-catenin is recruited by the T cell factor/lymphoid enhancer factor family (TCF/LEF) transcription factors regulating the expression of a large set of target genes. **b**, Enterotoxigenic *Bacteroides fragilis* (ETBF) and its associated metalloproteinase toxin, *Bacteroides fragilis* toxin (BFT), disrupt intestinal cell tight junctions and lead to the cleavage of E-cadherin, triggering a signalling cascade inducing *MYC* expression and sustained cell proliferation. ETBF lipopolysaccharide (LPS) also increases the expression of genes encoding several stemness transcription factors, such as sex determining region Y-Box 2 (*SOX2*) and Nanog homeobox (*NANOG*), via Toll-like receptor 4 (TLR4) signalling and through increased expression of JmjC domain-containing histone demethylase 2B (JMJD2B). **c**, The *Clostridioides difficile* virulence factor TcdB activates Wnt–β-catenin signalling. The mechanism for this is not completely known (dashed arrow). Through its glucosyltransferase domain, TcdB also induces necrosis through the assembly and activation of the NADPH oxidase (NOX) complex, leading to intracellular production of high levels of reactive oxygen species (ROS). **d**, *pks*$^+$ *Escherichia coli* produces the genotoxin colibactin, which induces interstrand cross-links and double-strand DNA breaks resulting in a specific and unique mutational signature. **e**, The *Fusobacterium nucleatum* Fap2 adhesin, important for *F. nucleatum* aggregative properties, interacts with D-galactose-β(1–3)-*N*-acetyl-D-galactosamine (Gal-GalNAc) sugar moieties. The FadA adhesin engages E-cadherin and induces cell proliferation via Wnt–β-catenin pathway activating target genes such as *MYC*, and contributes to a pro-inflammatory milieu. Via its LPS, *F. nucleatum* increases cancer cell proliferation and further activates the nuclear factor-κB (NF-κB) pro-inflammatory pathway. *F. nucleatum* also produces formate that engages aryl hydrocarbon receptor (AhR) signalling, increasing tumour invasion and cancer stemness through aldehyde dehydrogenase (ALDH) activity and induction of *SOX2*. CRC, colorectal cancer.

of intestinal tumours by producing butyrate[86]. This four-carbon short-chain fatty acid (SCFA) can inhibit the activation of NF-κB and reduce the secretion of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor (TNF), limiting pro-tumorigenic inflammation. Investigating the protective effects of specific microbial species against tumour growth can provide valuable mechanistic insights into how the gut microbiota contributes to maintaining a healthy gut and identify potential options for preventing and treating CRC.

Escherichia coli. *E. coli* is a gram-negative, facultative, anaerobic bacterium that is commonly found in the human gut. It is a relatively early colonizer in humans, often taking up residence during infancy[87]. With more than 700 serotypes identified, the vast majority of *E. coli* isolates are non-pathogenic. However, given the great interest in human diseases, there are numerous studies on *E. coli* that cause extra-intestinal and gut-associated disease[88,89].

E. coli strains that harbour the polyketide synthase (*pks*) pathogenicity island (fewer than 15% of all *E. coli*) can induce DNA damage in colonic epithelial cells via colibactin, a virulence factor that forms DNA cross-links and induces DNA double-strand breaks[14,90–92]. Colibactin biosynthesis is encoded by 19 *clb* genes, a gene cluster found in several members of the Enterobacteriaceae family (that is, *Citrobacter koseri*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*), which are all able to produce this genotoxin[93]. Recent studies have provided fundamental insight into the basic biology of how colibactin damages DNA, the nature of its induced DNA adducts, its associated mutational signatures in CRC and its regulation[94]. Using human intestinal organoids infected with colibactin-producing *E. coli*, a recent study by Pleguezuelos-Manzano et al. identified a colibactin-specific mutational signature[76]. The corresponding colibactin-associated mutational signature is characterized by single-base substitutions (SBS-pks), and a small indel (ID-pks) with deletions and insertions at T sites. The specific signature identified in the in vitro studies was detected in about 100 patients with CRC from a pan-cancer cohort of more than 5,000 patients[76]. The facts that *pks*$^+$ *E. coli* colonizes humans from early childhood, CRC takes many decades to develop and a colibactin signature is found in CRC underscore the need for thorough understanding of how colibactin is synthesized, how it damages DNA and how *E. coli* protects itself from colibactin-induced DNA damage.

pks$^+$ *E. coli* secretes small molecules known as 'precolibactins', some of which harbour a cyclopropane ring commonly seen in DNA alkylating agents[95]. Colibactin's reactive cyclopropane warhead accounts for its DNA alkylating ability which results in the DNA adducts that likely drive its mutational signature[92]. The *clb* gene cluster comprises a self-resistance protein (clbS) that binds and can deactivate colibactin, and represents one mechanism of many by which *pks*$^+$ *E. coli* may protect itself from colibactin-induced damage[96]. It is unclear whether targeted nuclear expression of clbS in human cell lines or colon organoids could afford protection against colibactin-induced DNA damage, but experimentally testing this idea is of translational interest.

Characterization of the colibactin biosynthesis pathway and the factors regulating its expression is important, as inhibiting colibactin directly or modulating its regulators could be cancer preventative or useful as a CRC treatment[96–98]. Briefly, the key enzymes of the pathway are the phosphopantetheinyl transferase ClbA, which activates the non-ribosomal peptide synthetase (NRPS) complex, ClbM that transports the precolibactin into the cytoplasmic membrane, and ClbP which induces the final colibactin maturation via its peptidase activity[99]. Recently, ClbR was also identified as a mediator of colibactin expression via transcriptional regulation[100]. Small molecule inhibitors have been identified that block the colibactin biosynthesis pathway[101] by inhibiting ClbP activity. HeLa cells infected with *pks*$^+$ *E. coli* and treated with the small molecule inhibitors show no advert cytotoxicity and very low off-target effects[101]. These promising results will require further validation in preclinical models. If validated, clinical trials may be challenging, as targeting a microbiota-derived toxin for cancer prevention for a disease such as colon cancer that takes decades to develop is time and resource intensive.

Campylobacter jejuni. *Campylobacter jejuni* is a gram-negative bacterium that can promote intestinal tumorigenesis via the production of cytolethal distending toxin (CDT), a genotoxin that causes DNA double-strand breaks[99]. In the $Apc^{Min/+}$ mouse model of intestinal tumorigenesis, a human clinical isolate of *C. jejuni* 81–176 potentiated carcinogenesis in a CDT-dependent manner[102]. Other *Campylobacter* spp. such as *Campylobacter concisus*, initially identified as an oral pathogen, are also associated with gastrointestinal tract diseases, including inflammatory bowel diseases (IBD) and CRC[103]. Metatranscriptomic analysis of CRC and adjacent normal mucosa revealed co-aggregation between *Campylobacter* spp., mainly *Campylobacter showae*, and species from the *Fusobacterium* genera in colon tumour versus healthy tissues[104]. The links between fusobacteria and CRC are explored further

Glossary

γ-H2AX
A sensitive marker of DNA damage, as phosphorylation of H2AX is required for the assembly of the DNA double-strand repair machinery.

Apc$^{Min/+}$ mouse model
Mice carrying a heterogeneous mutation in the commonly mutated (more than 80% of patients with colon cancer) adenomatous polyposis coli (*APC*) gene that develop spontaneous intestinal adenomas.

Azoxymethane (AOM)–dextran sodium sulfate (DSS) mouse model
A chemically inducible mouse model of colitis-associated colon cancer, where mice are treated with AOM, a jet fuel-derived mutagenic agent that damages the DNA of colonic epithelial cells, followed by three cycles of mucosal disruptant DSS.

Bacterial species
Bacteria sharing common genomic features and exhibiting a high degree of similarity in phenotype.

Bacterial strain
A genetic variant of a particular species of bacteria.

Bacteriophages
Viruses that infect and replicate within bacteria.

Biogeography
Localization at particular body sites.

Caecal microbiota transplant
(CMT). The transfer of caecal contents and microorganisms therein from a donor to a recipient host.

Faecal microbiota transplant
(FMT). The transfer of the microorganisms from the stool of a donor to a recipient.

Familial adenomatous polyposis
(FAP). A rare, autosomal dominant syndrome, involving the adenomatous polyposis coli (*APC*) gene, that predisposes an individual to tumours of the colon and rectum.

Genotoxic
A property that induces genetic damage (DNA mutation) within a cell.

Gnotobiotics
A specialized microbially controlled animal husbandry practice enabling experiments in which animals can be kept completely devoid of microorganisms or with defined microbial communities.

Gram-negative
A description of a bacterium that harbours an outer lipid membrane and does not retain crystal violet staining (Gram staining).

Gram-positive
A description of a bacterium that does not harbour an outer lipid membrane and thus retains crystal violet staining (Gram staining).

Microbiome
The collection of microorganisms (archaea, bacteria, fungi, protists and viruses) that inhabit a specific environment.

Mutational signature
The combination of mutations emerging from DNA damage and repair processes.

Oncomicrobe
Microorganisms with established features that influence cancer susceptibility and therapeutic response.

Symbionts
Organisms living in a neutral or beneficial way with their host.

Theranostic
The combination of therapeutics and diagnostics.

Type 3 secretion system
A bacterial complex or injectisome widely used by gram-negative bacteria to inject their effector molecules or toxins into host cells.

below and more studies are needed to understand how *Campylobacter* and *Fusobacteria* spp. may work cooperatively to promote carcinogenesis. In infectious diseases, many infections are polymicrobial and bacteria can influence one another, often with negative consequences for the host. This concept raises the question of how tumour-associated co-occurring bacteria may additively or synergistically affect tumorigenesis and metastasis.

Bacteroides fragilis. *B. fragilis* is a gram-negative anaerobe, with high intraspecies genetic diversity. Similar to *E. coli*, it is an early colonizer of the human gut[105,106]. *B. fragilis* strains are human gut symbionts that facilitate immune homeostasis within the CD4$^+$ T cell compartment[107]. However, ETBF expresses a toxin called *Bacteroides fragilis* toxin (BFT) which leads to a far different set of interactions with its hosts and contributes to both IBD and CRC pathology[108,109]. BFT has three different isotypes (BFT1, BFT2, BFT3)[110], and ETBF isolates that express *bft-1* and *bft-2* are frequently identified among ETBF CRC isolates[110,111]. Their detection is also associated with a poorer prognosis in some patient cohorts[110]. BFT is a 20 kDa matrix metalloproteinase that has direct effects on intestinal epithelial cells: it binds the extracellular domain of E-cadherin inducing activation of Wnt–β-catenin, *MYC* expression and NF-kB signalling pathways, triggering chronic inflammation[112–115]. ETBF also increases the expression of several stemness transcription factors such as sex determining region Y-Box 2 (SOX2) and Nanog homeobox (NANOG) via Toll-like receptor 4 (TLR4) signalling, and through increased JmjC domain-containing histone demethylase 2B (JMJD2B), suggesting that ETBF LPS may alter intestinal epithelial self-renewal and differentiation properties[116]. In patients with familial adenomatous polyposis (FAP), ETBF and *pks*$^+$ *E. coli*, measured by the presence of the *bft* and *clb* genes, were found to co-localize in tissue-associated patches or biofilms[117]. Co-colonization with ETBF and *pks*$^+$ *E. coli* was higher in biopsies of patients with FAP (52%, $n = 25$) as compared with healthy individuals ($n = 23$)[117]. In the *Apc*$^{Min/+}$ genetically engineered mouse model (GEMM) of CRC, ETBF triggers a pro-inflammatory TME through the induction of STAT3 in epithelial cells and subsequent accumulation of T helper 17 cells (T_H17 cells) and γδ T cells producing the pro-inflammatory cytokine IL-17 (ref. 78). Colonic regulatory T cells (T_{reg} cells) in this ETBF model play a pivotal role in driving an IL-17 pro-tumorigenic programme. Depletion of T_{reg} cells in ETBF-colonized *Apc*$^{Min/+}$ mice shifted the effector CD4$^+$ T helper response, resulting in an interferon-γ (IFNγ)-centric inflammatory response and an absence of tumorigenesis at early stages[13]. These studies are aligned with observations in the TME of human CRC, where increased numbers of CD4$^+$ T cells correlate with improved prognosis[118] and decreased effector T cell/T_{reg} cell ratios correlate with a worse prognosis[119].

Given that ETBF engages Wnt–β-catenin signalling and NF-κB pro-tumorigenic inflammatory pathways, investigators wondered whether there was a specific mutational signature associated with ETBF in CRC, akin to observations of *pks*$^+$ *E. coli* and CRC. Whole-exome sequencing combined with whole-genome sequencing (WGS) of tumours from

Apc$^{Min/+}$ mice with and without ETBF did not reveal a mutational signature unique to ETBF, and instead highlighted the overall very low level of mutagenesis in ETBF-colonized tumours[120]. Thus, despite the multifaceted functions of BFT and ETBF on host biology, epithelial cell mutagenesis cannot be ascribed to ETBF, or ETBF alone. These data highlight the range of effects that microbes can exert to promote carcinogenesis, whereas much still remains to be explored about bacterial toxins and the mechanisms by which they initiate colorectal carcinogenesis.

Peptostreptococcus anaerobius. *Peptostreptococcus anaerobius*, a gram-positive anaerobic bacterium enriched in faecal samples and mucosal tissue from patients with CRC, is an emerging CRC oncomicrobe[121,122]. Preclinical studies in the *Apc*$^{Min/+}$ mouse model of CRC colonized with *P. anaerobius* support that it potentiates tumorigenesis in vivo[123–125]. Long et al. identified a bacterial surface protein, putative cell wall binding repeat 2 (PCWBR2), that binds to the α2β1 integrin receptor expressed on CRC cells. α2β1 integrin can then recruit and activate non-receptor tyrosine kinases such as Src, which then promote focal adhesion kinase (FAK) phosphorylation and activate downstream phosphoinositide 3-kinase (PI3K)–AKT signalling, to both enhance cell proliferation and activate NF-κB. *P. anaerobius* also elicits an immune response notable for infiltration of myeloid-derived suppressor cells (MDSCs) in the TME of *Apc*$^{Min/+}$ mice[126]. MDSCs are pro-tumorigenic as they can compromise CD8$^+$ T cell antitumour immunity, are pro-angiogenic and can potentiate metastasis[127,128]. *P. anaerobius* also activates TLR2 and TLR4 signalling which increases intracellular reactive oxygen species (ROS) levels and supports cell proliferation through activation of cholesterol biosynthesis[129]. This is a characteristic of many cancer types and is vital for cell membrane biogenesis, cell survival and growth. It is also a precursor of many metabolites such as bile acids and sex hormones, that are increasingly recognized for their pro-tumorigenic effects[123,124]. Thus, similar to other oncomicrobes, *P. anaerobius* has pleiotropic effects on host cells, engaging with many aspects of cellular functions that are hallmarks of cancer (for example, genome instability and metabolism)[11,125].

Clostridioides difficile. *Clostridioides difficile* (formerly known as *Clostridium difficile*) is a gram-positive anaerobe and the leading cause of antibiotic-associated diarrhoea[130]. Investigating human CRC mucosal bacterial slurries using the *Apc*$^{Min/+}$ mouse model of CRC, Drewes et al. uncovered a pro-tumorigenic role for *C. difficile* through the activity of its toxin TcdB[83]. Mechanistically, TcdB induces activation of the Wnt–β-catenin pathway in crypt progenitor cells, as revealed by colon transcriptome profiling from slurry-gavaged *Apc*$^{Min/+}$ mice. Through its glucosyltransferase domain, TcdB also stimulates the NADPH oxidase (NOX) complex to produce ROS[131]. Activated myeloid cells within this TME led to the expansion of pro-tumorigenic IL-17-producing lymphoid cells[83]. It is important to note that the amount of *C. difficile* is low (less than 0.5% of the total microbial relative abundance within the slurry) in tumours of patients with CRC[132], and data linking *C. difficile* to human CRC remains very limited. As such, its role in CRC initiation or progression warrants further investigation in both preclinical models and human patient samples[133,134].

Morganella morganii. Given the connections between bacterial genotoxins and colonic carcinogenesis, Cao et al. screened more than 100 human gut microbes to identify genotoxic species or associated secreted metabolites that induce DNA damage in both cell-free and cell-based assays[135]. Small molecules, namely indolimines, induce cell-cycle arrest and DNA damage, as measured by γ-H2AX, a sensitive marker of DNA damage. *Morganella morganii*, a gram-negative bacterium enriched in both patients with IBD and patients with CRC compared with healthy individuals, produces indolimines. In a preclinical model of colitis-associated CRC, the azoxymethane (AOM)/dextran sodium sulfate (DSS) mouse model, the local colonization of *M. morganii* increases the tumour burden compared with the uncolonized control. *Clostridium perfringens* and *Clostridium ramosum* were also identified as genotoxic species by Cao et al., but the mechanisms by which these *Clostridium* spp. damage DNA and the relevance for human disease require further investigation.

Fusobacterium nucleatum. *Fusobacterium nucleatum* is a gram-negative anaerobe and one of the most abundant members of the oral microbiota[136]. For many decades, it was studied in periodontal diseases and in the placenta as a contributing agent to preterm birth[137,138]. Known as both an opportunistic pathogen and a bridging organism instrumental for dental plaque formation, *F. nucleatum* can interact and aggregate with many different bacteria via its elongated shape and its adhesins RadD, CmpA, FadA, Fap2 and FomA[139–141]. Through RadD, *F. nucleatum* binds to *Streptococcus mutans*, mediating their co-aggregation in biofilms, and to the yeast *Candida albicans* contributing to polymicrobial pathogenesis[142,143]. Although found in the mouth of healthy individuals, *F. nucleatum* has been associated with several types of oral and also extra-oral diseases such as appendicitis and pericarditis, and is even found in head and neck cancers, but its contribution to cancer initiation of these squamous cell malignancies remains unclear[136,144–147].

In the past decade, researchers found that *F. nucleatum* is also enriched in CRC tissues as compared with the adjacent normal tissue and may make its way from the mouth to the colon via a haematogenous route[148–151]. Several types of adenocarcinomas, including CRC, express high levels of D-galactose-β(1-3)-*N*-acetyl-D-galactosamine (Gal-GalNAc) sugar moieties at early and metastatic stages of disease[81,152,153]. *F. nucleatum* interacts directly with the host polysaccharide Gal-GalNAc through its adhesive lectin Fap2 (ref. 81). However, *F. nucleatum* has also been detected in samples isolated from CRC tissues, lacking Gal-GalNAc expression[154], supporting that it may have several ways by which it can adhere to neoplastic colonic epithelial cells. Given that *F. nucleatum* is detected in human lymph node, omental and liver metastases, many have wondered how *F. nucleatum* reaches metastases. These metastatic sites can express Gal-GalNAc. However, it remains unknown whether *F. nucleatum* reaches metastases by binding to the surface of metastasizing cells, living within metastasizing cells or through the bloodstream. Another route for dissemination of *F. nucleatum* to CRC metastases may stem from a recently described phenomenon called gut vascular barrier (GVB) impairment[155]. The GVB regulates bacterial dissemination from the gut to the liver. The *E. coli* virulence factor VirF when expressed by *E. coli* in colon tumours can increase dissemination of bacteria to the liver. Whether this route explains how *F. nucleatum* reaches liver metastases is not known. Beyond Fap2, another *F. nucleatum* adhesin, FadA, plays an important role in cancer initiation. FadA interacts with E-cadherin, leading to β-catenin translocation and expression of downstream Wnt– β-catenin target genes (for example, the genes encoding Myc and cyclin D1)[80]. Via its LPS, *F. nucleatum* increases cancer cell proliferation and activates the NF-κB signalling pathway promoting chronic inflammation[156]. In a TLR4-dependent manner, *F. nucleatum* LPS also induces the expression of microRNA-21 (miRNA-21), activating autophagy in CRC cells, further conferring chemoresistance[156,157].

The interactions between *F. nucleatum* and immune cells are integral to its tumorigenic effects. In mouse models of CRC, *F. nucleatum* drives a pro-inflammatory milieu, with intra-tumoural myeloid infiltration potentiating tumorigenesis[15]. *F. nucleatum* also impairs antitumour immunity via Fap2 that binds T cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT), an immune checkpoint inhibitor present on adaptive lymphocytes and natural killer (NK) cells, and inhibits NK cells cytotoxic activity[79].

F. nucleatum is metabolically active in evolving TMEs, and its metabolites can further contribute to its roles in carcinogenesis by altering the function of the immune system. *F. nucleatum* colonization in mouse models increases the levels of immunomodulatory SCFAs in the colon[158]. Formate, acetate, propionate and butyrate are SCFAs found within the human colonic luminal contents, and are produced by bacterial fermentation of dietary fibres and amino acids[159]. SCFAs can elicit numerous changes, often in a receptor-mediated fashion, in different types of immune cells such as T_{reg} cells, innate lymphoid cells type 3 (ILC3s), neutrophils and dendritic cells[160–164]. SCFAs can also influence intestinal epithelial cell production of cytokines and chemokines that activate and attract immune cells. SCFAs bind several G-protein-coupled receptors (GPCRs) including FFAR2 (GPR43), FFAR3 (GPR41) and HCAR2 (refs. 163,165,166). Brennan et al. found that *F. nucleatum* influences the intestinal immune landscape by increasing both colonic *IL17a* expression and colonic T_H17 cell numbers in an FFAR2-dependent manner[167] (Box 1).

Apart from the effects of its metabolites on the immune system, a recent study found that tumours with high levels of *F. nucleatum* display a metabolic shift towards glutamine metabolism[168]. Many cancer cells rely on glutamine, an abundant amino acid in the body, for their growth and division, as it serves as an important carbon source for nucleotide and fatty acid synthesis[169,170]. In mouse models of CRC, *F. nucleatum* produces high levels of the electron donor formate, a metabolic intermediate in one-carbon metabolism, that engages aryl hydrocarbon receptor (AhR) signalling, increasing tumour invasion and cancer stemness. The AhR pathway regulates cancer stem cell proliferation through aldehyde dehydrogenase (ALDH) activity and induction of *SOX2* gene expression[171]. Thus, *F. nucleatum* oncogenic features rely on many different mechanisms. By attracting tumour-permissive myeloid cells to the TME, inhibiting antitumour immunity and directly affecting host cell functions critical for cell proliferation and metabolism, the relocalization of *F. nucleatum* from the mouth to the colon is detrimental for its hosts as it creates a pro-tumorigenic and pro-metastatic TME.

F. nucleatum, in contrast with other oncomicrobes, does not encode known toxins, but can still potentiate CRC in several preclinical models and is consistently found in human CRC microbiome sequencing meta-analyses[151]. With time, researchers are likely to uncover more mechanisms by which *F. nucleatum* can trigger and shape the CRC TME. The challenges of the genetic manipulation of many CRC-associated human isolates of *F. nucleatum* strains and the lack of stable colonization in mouse models are crucial limitations that need to be overcome to decipher the roles of *F. nucleatum*, in concert with other bacteria, in CRC initiation and/or progression[172].

Pancreas

Pancreatic ductal adenocarcinoma (PDAC) is a gastrointestinal malignancy with a low 5-year survival that is often diagnosed at advanced stages[173]. Similar to CRC tumours, PDAC can harbour a diversity of microbial species including bacteria[55,56]. In epidemiological studies, oral dysbiosis with an increased abundance of *Porphyromonas gingivalis* and decreased abundance of *Streptococcus mitis* correlated with an increased risk for PDAC[174,175]. In a more recent study, Riquelme et al. observed that the microbiome composition of PDAC tumours correlated with intra-tumoural immune infiltration and survival[18]. Specifically, researchers identified an intra-tumoural microbiome signature that consisted of *Pseudoxanthomonas–Streptomyces–Saccharopolyspora–Bacillus* and correlated with improved outcomes; long-term survivors (LTS) of PDAC displayed increased intra-tumoural microbial diversity compared with individuals with shorter overall survival (STS). In the LTS group, Alphaproteobacteria, Sphingobacteria and Flavobacteria predominated, whereas the STS group was enriched for Clostridia and Bacteroidea. The PDAC LTS group also exhibited increased immune activation compared with individuals with STS, in whom the researchers noted increased levels of $CD4^+FOXP3^+$ T_{reg} cells and MDSCs, consistent with a pro-tumorigenic milieu (Fig. 2).

PDAC-residing bacteria can also inactivate chemotherapeutic drugs, leading to their reduced efficacy in killing pancreatic cancer cells[176]. The bacterial enzyme cytidine deaminase, primarily found in *Gammaproteobacteria* spp., can convert and inactivate gemcitabine, a chemotherapy drug commonly used to treat PDAC. High levels of these bacteria in patients' tumours were associated with a poorer response to chemotherapy and worse overall survival. These findings provide important mechanistic insights into how the gut microbiota may influence therapeutic outcomes in patients with PDAC and suggest potential targets for improving chemotherapy efficacy.

Enrichment of microorganisms within PDAC tissue samples is not restricted to bacteria, as fungi and other members of the microbiome have been associated with PDAC tumorigenesis. Aykut et al. uncovered that the mycobiome (fungal species) of PDAC tissue samples was enriched for *Malassezia* spp.[31]. These are complex fungi, found in about 90% of adults, that are part of the normal human skin microbiome of the scalp and face[177]. In PDAC tissue samples, *Malassezia* triggered tumour growth through the binding of mannose-binding lectin (MBL) by its fungal wall glycans, activating complement C3 cascade. Complement activation stimulates extracellular matrix remodelling within the TME as well as pro-tumorigenic signalling in tumour-associated macrophages and neutrophils[178]. Characterizing non-bacterial members of the microbiome in different cancers, such as fungi and viruses, is part of an emerging trend within microbiome sciences[32,179]. Whereas these new studies are often focused on characterizing what non-bacterial microbiome members are present, there is a crucial need to determine if and how these organisms directly contribute to tumorigenesis[31].

Lung

The human body comprises many microbial niches especially at its barrier surfaces (for example, the skin; Box 2). Many of these sites have lower carriage of microorganisms than the gastrointestinal tract and, as such, the roles of microbial–host cell interactions for tumorigenesis are just beginning to be appreciated. Recent research has uncovered a role for microbiota-driven cancer initiation and progression at body sites, such as the lung, previously considered to harbour very low or no microbial biomass in the absence of overt infection[1,2,17]. As a barrier site that interfaces with the external environment with every breath, the lung is susceptible to local inflammation triggered by infectious exposures, environmental allergens, pollutants and cigarette smoke. Non-small cell lung cancer (NSCLC), the most common type of lung cancer, is the leading cause of cancer-related deaths worldwide and deciphering the roles of all factors that contribute to its carcinogenesis

Box 1

The interaction between microbes, CD4$^+$ T cells and CD8$^+$ T cells in colon cancer

Both CD8$^+$ T cells and CD4$^+$ T cells play pivotal roles in antitumour immunity[259] and an increasing number of studies are revealing how gut microbes and gut microbial consortia influence the development, function and cell states of these lymphocytes[160,161,260,261].

CD4$^+$ T cells engage in reciprocal interactions with innate immune cells and secrete interferon-γ (IFNγ), tumour necrosis factor (TNF) and other cytokines and chemokines that activate cellular immunity[259]. CD4$^+$ T cells have brakes or regulatory checkpoint molecules, such as programmed death 1 (PD1). PD1 directly interacts with programmed death-ligand 1 (PDL1), which can be expressed on tumour cells, to inhibit immune activation.

In intestinal tissues, CD4$^+$ T cells are located in the lamina propria (LP) and subdivided into four major subtypes with distinct biological roles: T helper 1 cells (T_H1 cells), T helper 2 cells (T_H2 cells), T helper 17 cells (T_H17 cells) and regulatory T cells (T_{reg} cells)[262]. T_{reg} cells mediate immune tolerance, maintaining homeostasis in tissues[262]. Distinct T_{reg} cell subsets regulate the different types of effector T helper cells[263]. The roles of T_H17 cells in inflammation and cancer immunity are complex[264]. T_H17 cells secrete interleukin-17A (IL-17A) and IL-22. These cytokines have pleiotropic effects and play important roles in host defence and epithelial barrier maintenance. They are also implicated in the pathogenesis of autoimmune diseases and several malignancies, with roles in both cancer initiation and progression[265,266]. CD4$^+$ T cell polarization into T_H17 cells can be shaped by several bacteria such as segmented filamentous bacterium (SFB; *Candidatus Savagella*), enterotoxigenic *Bacteroides fragilis* (ETBF), *Bifidobacterium* spp., *Fusobacterium nucleatum* and some fungi[13,167,260,267,268]. Several preclinical studies have uncovered pro-tumorigenic roles for T_H17 cells in the tumour microenvironment (TME), associated with an inflamed and tolerogenic milieu[13,78,167,260,266,269]. In contrast, the presence of both T_H1 cells and T follicular helper cells are associated with improved antitumour immunity. More specifically, T_H1 cell activation and numbers correlate with improved prognosis[118,269]. Conversely, the accumulation of T_{reg} cells in the TME correlates with a worse prognosis, in line with their role in reducing the immune response and inhibiting the cytotoxic functions of CD8$^+$ T cells[119,270].

CD8$^+$ T cells and CD4$^+$ T cells work together to promote antitumour immunity. CD4$^+$ T cells recognize antigens presented by major histocompatibility class II (MHC II) on dendritic cells. CD4$^+$ T cells then become activated and secrete cytokines (for example, IL-2, IFNγ and TNF) which are essential for CD8$^+$ T cell effector function, proliferation and survival[259]. In addition to cytokine secretion, CD4$^+$ T cells can directly activate CD8$^+$ T cells through the engagement of co-stimulatory molecules such as CD40L on the surface of CD4$^+$ T cells and CD40 on the surface of CD8$^+$ T cells. Dendritic cells directly activate CD8$^+$ T cells by presenting antigens via major histocompatibility class I (MHC I) molecules. Dendritic cells also internalize extracellular proteins, usually loaded onto MHC II molecules, and can present these as peptides on MHC I molecules to CD8$^+$ T cells in a process called 'cross-presentation'.

Given the importance of cytotoxic CD8$^+$ T cells for antitumour immunity, there is tremendous interest in identifying microbes, microbial consortia and microbial features that tune their tumour-fighting function. Tanoue et al. found that a consortium of 11 bacterial strains induced a strong CD8$^+$ T cell response that boosted the efficacy of immune checkpoint blockade in mice[261]. Other species such as *Enterococus hirae*, a gram-positive bacterium, promote antitumour immunity in mice by enhancing CD8$^+$ T cell antitumour responses when used in combination with cyclophosphamide chemotherapy[19,271]. Bachem et al. discovered that butyrate, a microbiota-derived short-chain fatty acid (SCFA), enhances CD8$^+$ T cell metabolism and promotes their differentiation into memory T cells[272]. Of note, SCFAs can also modulate the activity of dendritic cells and macrophages through the SCFA receptor GPR43, leading to enhanced CD8$^+$ T cell priming and proliferation[164]. Collectively, these findings suggest that microbial metabolites play important roles in guiding the function and metabolic rewiring of activated CD8$^+$ T cells. Other components of the microbiota and their secreted small molecules or the absence of beneficial microbiota components can have pro-tumorigenic function through modulation of CD8$^+$ T cells. Specifically, microbial components can directly or indirectly compromise CD8$^+$ T cell function, by eliciting exhausted responses and dampening antitumour immunity[164,273]. This can be driven by CD8$^+$ T cell over-activation, immunosuppression or a lack of stimulatory inputs. Further studies are necessary to decipher the effects of the microbiota on CD8$^+$ T cell function in colorectal cancer (CRC) tumorigenesis.

and response to treatment is of the utmost public health import[180]. Moreover, the lung microbiome is emerging as a potential contributor to lung cancer[181].

The exact contribution of the lung microbiome to NSCLC is currently understudied, and several studies suggest that few viable microbial cells can be isolated from healthy lungs, either due to a low biomass or to technical detection limitations[182–184]. However, more than half of all patients with NSCLC have a recent history of bacterial pneumonia or other pulmonary infection[185]. Epidemiological studies have also revealed a strong association between *Chlamydia pneumoniae* infection, induction of chronic inflammation and tumorigenesis in the lung[186]. In NSCLC tissues, the presence of specific taxa correlates with oncogenic transcriptome programmes such as activation of the ERK and PI3K signalling pathways[187] (Fig. 2). This was further validated by exposing airway epithelial cells to bacteria such as *Prevotella*, *Streptococcus* and *Veillonella*, in vitro and in vivo, which lead to PI3K and AKT signalling activation. The enrichment of oral bacteria in lung parenchyma and their ability to trigger pathways contributing to

early stages of host cell transformation may provide novel avenues for investigation in lung cancer.

Apart from molecular epidemiological association studies using human tissues, preclinical models have also been utilized to uncover the mechanisms by which the microbiota can potentiate lung cancer tumorigenesis. Jin et al. found that depletion of the microbiota with an antibiotic cocktail in a lung adenocarcinoma mouse model harbouring *Kras* mutation and *p53* deletion (termed the KP model) significantly suppressed lung tumour growth[188]. More specifically, they found that a dysbiotic lung microbiota (imbalance between symbionts and pathogens) induced a pro-inflammatory and pro-tumorigenic tumour milieu with stimulation of IL-17-producing γδ T cells. Analysis of the bronchoalveolar lavage fluid using 16S rRNA gene amplicon profiling from tumour-bearing KP mice revealed a significant increase in bacterial burden notable for taxa from the *Herbaspirillum* genus and the Sphingomonadaceae family. In this study, activation of TLRs by microbial products (for example, LPS and peptidoglycan) led to activation of alveolar macrophages and neutrophils, elevated levels of tissue IL-1β and IL-23, and increased numbers of activated lung-resident γδ T cells[188].

Detection of microorganisms in organs previously considered sterile or of low biomass, in the absence of infection, may be a harbinger for loss of immune-microbial homeostasis and a contributor to chronic inflammation, known to increase the risk of cancer. Persistent dysbiosis during tumour development and progression can alter the immune system, influencing patient outcomes. In the case of NSCLC, chronic inflammation is a recognized and important risk factor for cancer development, and therefore further mechanistic studies are needed to decipher the contribution of the microbiota to its initiation and progression.

Sex-specific organs

Breast

Breast cancer is the most frequent cancer type in women, and a highly heterogenous disease in its molecular subtyping and response to treatment. The gut microbiota, via the 'estrobolome' (bacterial genes whose products are capable of metabolizing oestrogen, further described in Box 3), can regulate the levels of circulating free oestrogen and promote their reabsorption[189,190]. The accumulation of endogenous oestrogens can further contribute to an increased risk of developing breast cancer[191,192].

Other circulating small molecules derived from microbial metabolites, namely SCFAs and lithocholic acid (LCA), also function in tumour development and metastatic spread, with lower levels measured in patients with breast cancer[193–195]. LCA is a secondary monohydroxy bile acid that is generated from primary bile acids by microbial enzymes[196]. In contrast with its pro-tumorigenic roles identified in CRC and liver cancer[197,198], Mikó et al. found that LCA biosynthesis was downregulated in patients with breast cancer, mainly via a reduced abundance of *baiH*,

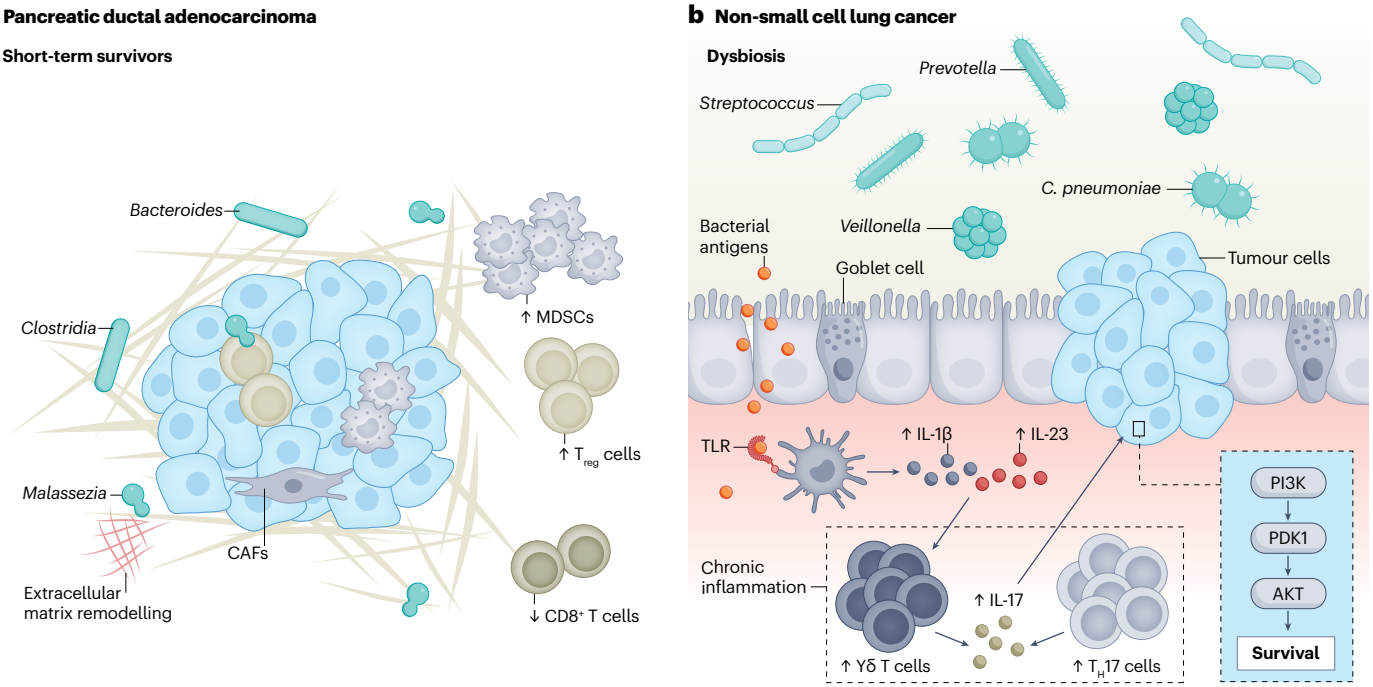

Fig. 2 | Bacteria-associated tumorigenesis in the pancreas and lung. Although previously considered sterile, recent work supports a role for the microbiota in cancers of the pancreas and lung. **a**, In pancreatic ductal adenocarcinoma (PDAC), tumour development has been associated with oral dysbiosis with an increased abundance of *Porphyromonas gingivalis* and decreased abundance of *Streptococcus mitis* when compared with healthy individuals. Composition of a pancreatic tumour's microbiome from patients with short-term survival (enriched in *Clostridia* and *Bacteroides*) correlates with intra-tumoural infiltration of myeloid-derived suppressor cells (MDSCs) and regulatory T cells (T_{reg} cells; $CD4^+FOXP3^+$ T cells), as well as a decrease in cytotoxic $CD8^+$ T cells. **b**, In non-small cell lung cancer (NSCLC), enrichment of specific species such as *Chlamydia pneumoniae* and genera (*Prevotella*, *Streptococcus* and *Veillonella*) can lead to direct upregulation of phosphoinositide 3-kinase (PI3K)–phosphoinositide-dependent protein kinase 1 (PDPK1; also known as PDK1)–AKT signalling. Lung microbiota ligands can increase levels of interleukin-1β (IL-1β) and IL-23 from myeloid cells, and activate and expand lung-resident T helper 17 cells (T_H17 cells) and γδ T cells, driving inflammation and further promoting tumour growth. CAFs, cancer-associated fibroblasts; TLR, Toll-like receptor.

the bacterial gene encoding the enzyme 7α/β-hydroxysteroid dehydroxylase. *baiH* can be expressed by different species (for example, *Clostridium sordelli*, *Staphylococcus haemolyticus*, *E. coli*) and encodes the key enzyme responsible for LCA production[194,199]. Mechanistically, LCA treatment in vitro and in vivo induces oxidative phosphorylation (OXPHOS) in breast cancer cells, inhibits epithelial-to-mesenchymal (EMT) transition and boosts antitumour immunity. LCA effects are mediated by G-protein-coupled bile acid receptor 1 (GPBAR1; also known as TGR5)[200]. Shotgun metagenomic sequencing analysis of faecal samples from patients with breast cancer revealed a negative correlation between *baiH* and thus LCA levels with both cancer prognosis and response to chemotherapy[201]. Microbiome-focused metagenomics and metabolomics has uncovered a role for gut microbe-derived metabolites (for example, SCFAs and LCA) in breast cancer, supporting the systemic effects of the gut microbiota in cancer progression (Fig. 3).

Microbe-derived small molecules are not the only mechanism by which the microbiota are associated with breast cancer progression. In a study by Parhi et al., the researchers hypothesized that *F. nucleatum* might localize to breast cancers as it does in CRC, via a haematogenous route, dependent upon neoplastic tissue sites expressing Gal-GalNAc sugar residues[17,81]. Gal-GalNAc levels are higher in human breast tumour samples when compared with matched benign tissue[202]. In vitro and in vivo experiments in the orthotropic 4T1 BALB/c mouse mammary cancer model revealed that *F. nucleatum* colonizes and potentiates tumorigenesis and reduces $CD4^+$ and $CD8^+$ T cell infiltration in a Fap2-dependent manner[17]. Mice colonized with *F. nucleatum* exhibited larger lung metastases than those in the sham-infected mice. Although *F. nucleatum* DNA has been detected in human breast cancer tissue[14], understanding the mechanisms by which it enhances tumour progression and metastatic spread will likely be important not only for breast cancer but also for other cancer types.

Prostate

Prostate cancer is the second most frequent cancer in men worldwide, and age, race and family history are major risk factors[203]. Importantly, diet and physical activity play a role in tumour development and progression and are mainly associated with the race differences found across incidence rates[204]. The standard of care and first-line treatment for prostate cancer is androgen deprivation therapy (ADT) as androgen receptor (AR) signalling and its abnormal activation is the main dysregulated pathway implicated in prostate cancer tumorigenesis. Although several studies suggest that there is a prostate-related microbiota[205,206], recent studies have focused on the roles of specific microbial species and their bioactive molecules in prostate cancer progression[207–210]. Matsushita et al. found that treatment with microbe-derived SCFAs upregulated the expression of insulin-like growth factor 1 (IGF1) and its receptor (IGF1R) in prostate cancer cells, further activating the MAPK and PI3K signalling pathways[211]. IGF1 is a growth factor that promotes the growth and survival of many types of cancer cells, including prostate cancer cells. Inhibition of the IGF1 pathway reduced the SCFA tumour-promoting effects in a mouse prostate cancer xenograft model. Using shotgun metagenomic analysis, a recent study established a link between sustained tumour growth through androgen biosynthesis by specific gut microbial species such as members of the *Ruminococcus* genera[209]. Pernigoni et al. found that specific *Ruminococcus* isolates were enriched in patients with castration-resistant prostate cancer (CRPC) and that these bacteria were able to synthesize dehydroepiandrosterone (DHEA) from pregnenolone, a precursor of testosterone (Fig. 3). Faecal microbiota transplant (FMT) from patients and mice with CRPC to recipient prostate cancer-harbouring mice led to the emergence of CRPC. Conversely, FMT from patients with hormone-sensitive prostate cancer controlled tumour growth in CRPC-bearing mice. The microbiota of patients with hormone-sensitive prostate cancer was enriched for species that belong to the *Prevotella* genus. Additionally, Terrisse et al. found that a higher diversity of the gut microbiota is associated with a more favourable response to ADT[210]. Specific bacterial strains, such as *Akkermansia muciniphila*, found at lower abundance in patients with prostate cancer prior to treatment (and thus enriched upon ADT), may contribute to the antitumour effects of ADT by promoting immune cell infiltration into tumours[210,212,213]. Collectively, these data suggest that specific bacteria are important influencers of prostate cancer progression and treatment response, highlighting the far-reaching and fascinating systemic effects of the gut microbiota.

Box 2

The cutaneous microbiome in skin cancers

The cutaneous microbiome contains millions of microorganisms and is gaining attention for its role in skin cancer[274,275]. The skin is the largest organ of the human body, and features several distinct environmental landscapes across the body (for example, scalp, nose, axilla and feet) that shape the composition of its microbial communities[276]. Studies led by the Belkaid, Grice, Kong, Segre and Knight laboratories describe and demonstrate the importance of the skin microbiota in regulating tissue homeostasis and immunity[277–282]. However, only a few studies have begun to decipher the role of the skin microbiota in shaping tumorigenesis and skin microbiota crosstalk with the immune system, both locally and distally[283].

A recent study by the Samuels laboratory found that melanoma cells present unique peptides to the immune system via their major histocompatibility complex class I (MHC I) and class II (MHC II) molecules derived from intra-tumoural bacteria, such as *Fusobacterium nucleatum*, *Staphylococcus aureus* and *Staphylococcus capitis*[217]. Using 16S rRNA gene sequencing with human leukocyte antigen (HLA) peptidomics from matched primary and metastatic tumour of patients, bacteria-derived peptide fragments can be identified in the groove of MHC molecules. Tumour-infiltrating lymphocytes, stimulated by these bacteria, triggered the efficient production of interferon-γ (IFNγ). This study demonstrates that intra-tumoural bacteria are a class of antigens that can serve as effective targets for immunotherapy.

Cancer prevention

Microbiome studies in cancer research have seen significant progress in recent years due to the advancements in detection methods for microbial entities and microbe-derived small molecules (Box 4). Additionally, modulation of the microbiome through targeted removal of the cancer-instigating oncomicrobes and their associated small molecules, or through enrichment of the microbes that improve antitumour immunity, holds tremendous potential for both cancer prevention and treatment.

Early detection

With its large number of studies focused on the interplay between the gut microbiota and tumour progression, CRC is a suitable model disease to investigate novel strategies for early cancer detection. Stool-based screenings are widely used for CRC screening and are an attractive, non-invasive approach compared with colonoscopies, especially in more resource-limited medical care settings[214]. The US Food and Drug Administration (FDA) has approved three types of stool tests: guaiac-based faecal occult blood testing (gFOBT), a faecal immunohistochemical test (FIT or iFOBT) and multi-target stool DNA testing (FIT-DNA)[215]. The gut microbiome holds diagnostic and prognostic implications for CRC as well as many cancer types, potentially paving the way for stool-based tests in other gastrointestinal tract-associated cancer types, for example PDAC and non-gastrointestinal tract malignancies[17,22,209,216,217]. For example, using shotgun metagenomics and 16S rRNA gene amplicon sequencing of faecal and salivary microbiota, both sample types showed potential for PDAC early detection in a Spanish case–control study[216]. At-home stool collection and testing kits are a promising strategy for early cancer detection and are already widely employed in Europe, especially in the United Kingdom for stool blood detection.

Chemoprevention and dietary modulation

Knowing the microbial instigators of cancer can provide insights into potential targets for cancer prevention strategies. The efficacy of cancer prevention strategies focusing on lifestyle, such as diet and medication, are now being investigated for how their benefits may be modulated by the microbiome[218]. Aspirin is a well-established chemopreventive agent for CRC[219]. Inhibition of Ptgs2 (COX2), which facilitates generation of inflammatory prostaglandins, and of NF-kB signalling and Wnt–β-catenin all underpin aspirin's chemopreventive effects[220,221] Although many epidemiological studies have shown that low-dose aspirin (81 mg) decreases the risk of CRC, its precise role in altering the human gut microbiota or whether its CRC-attenuating effects are modulated by the gut microbiome is still unclear, thus more mechanistic and clinical studies are warranted[219,222,223].

Both aspirin and its primary metabolite salicylic acid influence *F. nucleatum* growth and gene expression[224], in line with previous findings showing altered growth and transcriptome changes for other bacteria species[225–230]. In a recent study by Brennan et al., researchers showed in the $Apc^{Min/+}$ mouse model of CRC that *F. nucleatum*-associated colonic tumorigenesis could be entirely blocked by aspirin-supplemented chow, potentially by decreasing the pro-tumorigenic adhesins of *F. nucleatum*[224]. Although additional studies are required to understand aspirin-associated microbial vulnerabilities, this collective work is an example of how chemopreventive agents may be deployed in the future in a microbiome-informed manner.

Diet shapes the microbiome, and dietary variations can induce temporary microbial shifts within the microbiome and its metabolites[231,232]. Certain dietary patterns are associated with increased risk for CRC, such as high red meat or alcohol consumption[233–235].

Box 3

Microbiota and sexual dimorphism in disease

Both biological sex and sex hormones can influence tumour initiation, and sex hormones and their metabolism are influenced by the gut microbiota[284]. Sex hormones (androgens, oestrogens and progestogens) are steroids, derivatives of cholesterol, that are synthesized in the gonads and adrenal cortex by a series of enzymatic reactions involving two classes of enzymes; cytochrome P450s (CYPs) and hydroxysteroid dehydrogenases (HSDs)[285]. CYPs mediate hydroxylation and cleavage of the carbon–carbon bond, whereas HSDs catalyse the oxidoreduction of the hydroxy and keto groups in a nicotinamide adenine dinucleotide (phosphate) (NAD(P)H)-dependent manner.

Sexual dimorphism in immunity has been investigated for many decades, and whereas most findings point towards the role of sex hormones as drivers of this disparity, their modulation by the gut microbiota has also emerged as a potential key player[286]. In a landmark paper by Markle et al., the researchers found that in the non-obese diabetic (NOD) mouse model (which spontaneously develops type 1 diabetes), female mice display an increased susceptibility to disease compared with their male littermates[284]. However, when these mice were re-derived in the absence of microbes and maintained under germ-free conditions, the sex bias between male and female NOD mice disappeared. Caecal microbiota transplant (CMT) from male to female NOD mice prior to the disease onset was also protective against inflammation in pancreatic islets and the development of diabetes. In addition, the female NOD mice exhibited increased testosterone levels post CMT, and inhibiting the androgen receptor (AR) was sufficient to abolish the protection. This study helped establish the concept of crosstalk between the microbiota, sex hormones and the sex-specific risk for developing disease (for example, autoimmune diseases)[287].

This recognition of the connectivity between the gut microbiome and sex hormones led Plottel and Blaser to describe the concept of the 'estrobolome', in which the gut microbiome contributes to health and disease via "an aggregate of enteric bacterial genes whose products are capable of metabolizing oestrogen"[288]. Enzymes expressed by gut bacteria can modulate the levels of both oestrogen and testosterone, as well as their enterohepatic circulation[286]. Many gut bacteria harbour β-glucuronidases (encoded by *gus* genes) and β-glucuronides, two enzymes involved in the metabolism of oestrogen via deconjugation and conjugation[189,190]. The abundance of *gus* genes in the human microbiome suggests that the gut microbiota may play an important role as risk factors for sex-specific malignancies such as breast, ovarian, endometrial and prostate cancer[284,288,289]. Sex differences have also been described in bidirectional interactions among hormones, the microbiota and disease susceptibility, a concept termed the microgenderome[286]. Building on these seminal findings, the gut microbiota is now being investigated for its roles in modulating tumour progression and response to cancer treatment in the context of sexual dimorphism in cancer[290].

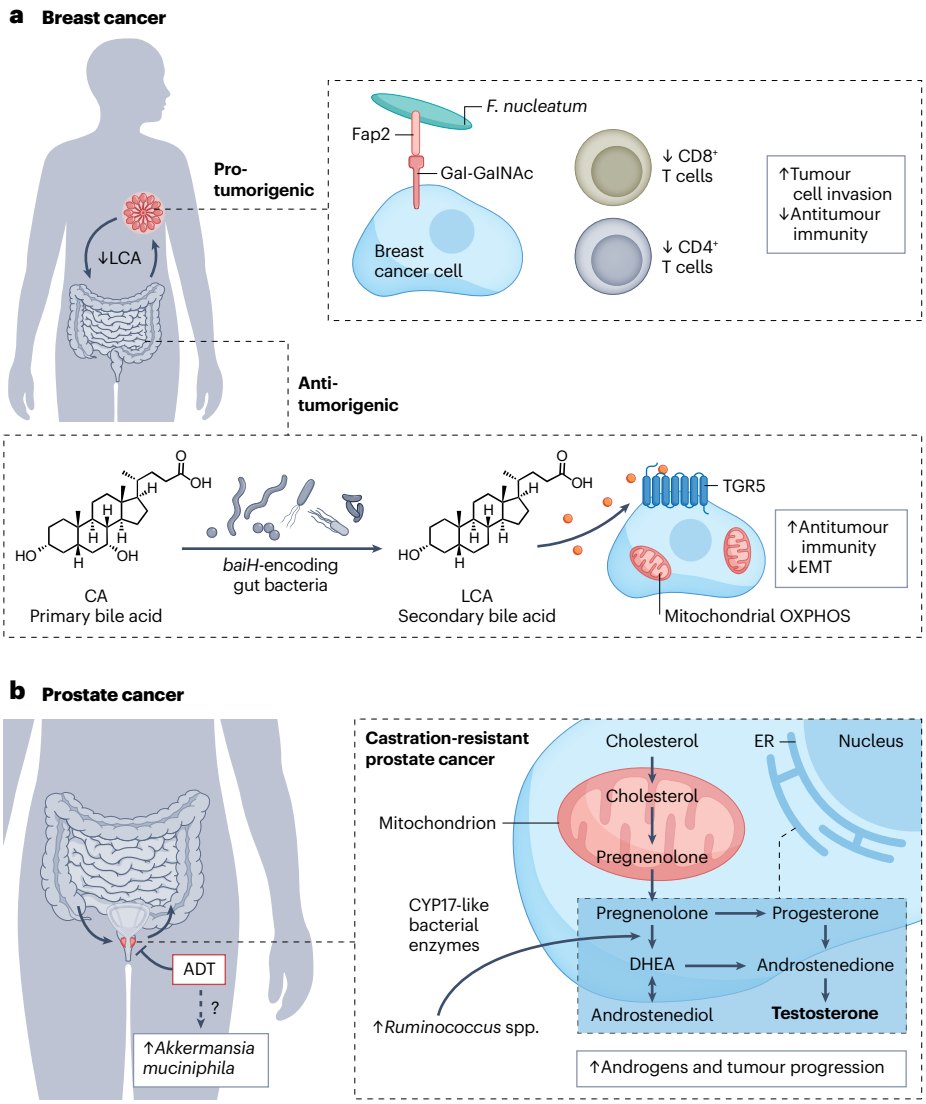

Fig. 3 | Microbiota tumour-associated features in breast and prostate cancer. Sex hormones (androgens, oestrogens and progestogens) are derivatives of cholesterol that are synthesized in the gonads and adrenal cortex by a series of enzymatic reactions. Metabolism of sex hormones can be influenced by the gut microbiota. **a**, In breast cancer preclinical models, *Fusobacterium nucleatum* can colonize tumours, potentiate tumorigenesis and reduce T cell infiltration. Short-chain fatty acids (SCFAs) and lithocholic acid (LCA) are microbe-derived intestinal metabolites that are downregulated in tissue samples. *baiH* is a bacterial gene encoding the key enzyme in LCA production from primary bile acids, such as chenodeoxycholic acid (CA). LCA induces oxidative phosphorylation (OXPHOS) in breast cancer cells through TGR5, inhibits epithelial-to-mesenchymal (EMT) transition and boosts antitumour immunity. **b**, In prostate cancer, *Ruminococcus* spp. are enriched in both preclinical models and patients with castration-resistant prostate cancer (CRPC) that relapsed after treatment with androgen deprivation therapy (ADT). *Ruminococcus* spp. synthesize dehydroepiandrosterone (DHEA) from pregnenolone, leading to increased testosterone levels in the bloodstream. Administration of *Ruminococcus gnavus* increases tumour growth in prostate cancer mouse models. Distinct gut microbiota members, such as *Akkermansia muciniphila*, are found at lower abundance in patients with prostate cancer, but are enriched upon ADT and may contribute to the antitumour effects of ADT by promoting immune cell infiltration into tumours. ER, endoplasmic reticulum; Gal-GalNAc, D-galactose-β(1–3)-*N*-acetyl-D-galactosamine.

In a large prospective study by Mehta et al., researchers studied the potential link between diet, specifically dietary fibre intake, and CRC incidence, finding that a high-fibre diet lowered the risk of developing *F. nucleatum*-positive CRC[236]. Given this, mechanistic explorations of how diet–microbiome interactions influence cancer susceptibility are warranted.

Treatment opportunities

Phage-based therapy

An exciting therapeutic strategy for selective bacterial targeting involves bacteriophages, which are viruses that can invade bacteria and are the most abundant members of the gut virome[237,238]. The potential therapeutic utility of naturally occurring bacteriophages emerged more than 100 years ago, prior to the current multidrug resistance crisis facing antibiotic use and infectious disease treatment[238,239]. Phage-based therapy has gained attention for its ability to precisely target both highly drug-resistant bacteria and oncomicrobes, without disrupting the homeostasis of the microbiome, unlike traditional antibiotics. Treatment with these viruses has led to promising results in several preclinical studies, such as the use of a specific bacteriophage targeting *H. pylori*[240,241]. Phages can also carry payloads that are released within the TME. Zhang et al. developed a phage-based strategy to both eliminate *F. nucleatum* and reduce side effects due to untargeted drug delivery (accumulation of drug in normal tissue rather than the tumour itself). They isolated a phage strain from human saliva that could specifically lyse *F. nucleatum* and engineered it to carry and deliver irinotecan, a chemotherapeutic drug used in CRC[242]. They also encapsulated the engineered phage in dextran particles, as bacterial members of the microbiome can metabolize dextran to SCFAs, which have potential benefits to the host and microbiota[242]. In preclinical mouse models, the administration of this therapeutic led to elimination of intra-tumoural *F. nucleatum* and reduced tumour growth[242]. Although it is in the early stages, phage-based targeting of oncomicrobes represents an exciting therapeutic avenue, and requires better understanding of resistance mechanisms as well as effects on the host immune system.

Engineered bacteria

Bacteria-based cancer therapy is a fascinating application from the field of synthetic biology, offering many opportunities for cancer care. The use of tumour-targeting bacteria as delivery vectors can increase the specificity of drug targeting and reduce toxicity to the patient. Bacteria can also preferentially reach necrotic or hypoxic areas of tumours which other treatments struggle to access because of compromised tumour vasculature[243]. *Salmonella enterica Typhimurium*, *Lactococcus lactis* and *E. coli* Nissle (EcN) are all used in the development of engineered bacterial cancer therapies[244–249]. EcN strains have been modified to modulate tumour metabolism or enhance antitumour immunity through activation of the stimulator of interferon genes (STING) pathway or inhibition of the common immune checkpoint receptors programmed death 1 (PD1), programmed death ligand 1 (PDL1) and cytotoxic T lymphocyte associated protein 4 (CTLA4) (refs. 250–252). Canale et al. engineered EcN to convert ammonia, a metabolic waste produced found in the TME, into L-arginine[251], a necessary metabolite for effective T cell-mediated antitumour immunity[253]. Using CRC preclinical models, colonization with this specific strain of EcN increased T cell infiltration and synergized with anti-PDL1 treatment. This highlights the possibility of combining microbial and immune system targeting therapeutics in cancer care. For additional recent reviews on the use of engineered bacteria for cancer, please see other recent publications[244,246,249].

Extracellular membrane vesicles

Healthy human cells, cancer cells and bacterial cells (mostly gram-negative bacteria) can all produce extracellular vesicles. These packets of cellular contents are referred to as extracellular membrane vehicles (EVs). EVs are a heterogeneous group of small membranous structures released by cells that can transport various small molecules, including nucleic acids, proteins, lipids and metabolites[254]. Although cancer cell EVs have been studied for their potential use in liquid biopsies for cancer detection[255], recent research highlights the role of microbial EVs in microbiota–host interactions and translational opportunities. For example, EVs derived from genetically modified *E. coli* are able to induce highly effective IFNγ-mediated antitumour responses and suppress tumour growth in CRC mouse models[256]. The potential roles of EVs not only for cancer aetiopathogenesis but also as cancer theranostics merit further inquiry[257,258].

Perspectives, future challenges and conclusions

The role of the microbiota in tumorigenesis has garnered considerable attention over the past two decades, yet the field remains full of correlative observations and associations that massively outnumber the field's mechanistic studies. Preclinical models that more faithfully recapitulate human cancer genetics and the human microbiome are now available with advancements in humanized gnotobiotic mouse models. However, human diet and environmental exposures remain underexplored variables in such studies. It is not only the models for study that present challenges but also the collection of patient materials for microbiome studies, especially for tumours that harbour low microbial biomass. The time required to collect surgical tumour specimens and preserve them may lead to the loss of specific bacteria, such as obligate anaerobes that die in the presence of oxygen.

Box 4

Technologies for assessing the microbiome in cancer

Oncomicrobes including species implicated in modulating cancer therapy responses can be detected through numerous techniques. For example, polymerase chain reaction (PCR) targeting the 16S rRNA gene provides rapid detection and quantification of specific species. 16S rRNA gene amplicon sequencing and other amplicon-based sequencing methods provide taxonomic information, often to the species level. Shotgun metagenomics sequencing helps identify and profile many microorganisms, providing insight into both the composition and the functional capacity of a microbiome. This technique lacks a microbial directed amplification step. Thus, in tumours which are abundant in host cells and their DNA, it can be costly to attain the sequencing depth required for detecting ample microbial reads.

Visualizing the interactions between both microbial communities and host cells within human tissues is increasingly possible with current technologies. Studying the presence and distribution of microorganisms in tumours provides insights into their spatial distribution and functional roles in the tumour microenvironment (TME). Microorganisms can have different roles depending on which cell type they are able to interact with, bind to or access within the TME. As evidenced by research on the various cells within the TME, cellular function can vary depending on their spatial distribution in the tumour[291]. Researchers have developed imaging-based spatial transcriptomics, a technology that measures both the copy number and the spatial distribution of RNA species in single cells, allowing for gene expression profiling in a range of biological samples[292]. Similarly, gene expression profiles of individual bacteria and their physical distribution within a defined structured environment (for example, a tumour) can also be studied. Such studies employ high phylogenetic resolution by fluorescence in situ hybridization (HiPR-FISH) and parallel sequential fluorescence in situ hybridization (par-seqFISH)[293,294]. Pro-tumorigenic expression changes in the host cells can be linked to particular microbiota enrichments with distinct spatial architectures[295]. Galeano Niño et al. identified microbial species, their corresponding localization and the related molecular changes triggered in the host cells, within patient tissues. This study is helping establish the foundation for spatial transcriptomics in investigating host–microbiota interactions in the TME.

Ultimately, the detection of oncomicrobe features associated with impaired antitumour immunity or carcinogenesis (for example, Fap2 for *Fusobacterium nucleatum*, colibactin for *pks*$^{+}$ *Escherichia coli*) may be more useful clinically and therapeutically than the detection of a given bacterial species itself. Virulence factor discovery efforts and the development of new methods should be geared to efficient, low cost and effective virulence factor detection. Such data will ultimately provide critical information for the establishment of microbial biomarkers that guide diagnosis, prognosis and therapy in cancer treatment.

Additionally, when a tissue is frozen or embedded in paraffin, it can become contaminated with environmental, non-tumour-specific microbes. This, as well as misidentifying microbial reads and over-calling microbial reads, are substantial problems for microbiome scientists. Developing methods to address these problems of sorting bona fide versus spurious microbial signals from samples remains a challenge for the field.

Many of the recent findings discussed in this Review have shed light on microbiome–cancer interplay, some of which pave the way for promising novel cancer therapies and provide insights into the basic biology of microbiota-associated cancer initiation and progression. However, much remains to be discovered for the field to progress. With improvements in detection methods for microbial entities and the microbe-derived small molecules by which their exert effects on human biology, microbiome sciences hold great potential for cancer prevention, detection, diagnosis and treatment.

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