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Erle S. Robertson *Editor*

Microbiome and Cancer

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Preface

Studies targeted to understanding our interactions with the microbial world have been ongoing for more than a century. This was demonstrated through the initial link between infectious agents and cancer, as identified by Peyton Rous. He showed an association between a filterable agent and development of sarcomas in chickens, in 1911. This agent was identified as the Rous sarcoma virus and was shown to be transferrable to birds that were disease free. Approximately five decades later, the first human oncogenic virus was identified by Anthony Epstein and Denis Burkitt, with colleagues Yvonne Barr and Bert Achong, in 1964 at the Middlesex Hospital in England. This certainly changed our understanding of the contributions of infectious agents to the cancer phenotype many years after the discovery of the first link between cancer and the RSV agent in avian species. Today there have been increasing associations with infectious agents and human cancers from viruses to parasitic agents. In fact, two of the most impressive successes in the cancer vaccine arena have been against viral agents, as seen with vaccines against the hepatitis B virus (HBV) and the human papilloma virus (HPV). The effectiveness of these vaccines in reducing the incidences of hepatocellular cancer (HCC) and cervical cancer, respectively, demonstrates the importance of understanding the links between pathogenic infectious agents and cancer.

Today approximately 20% of all known cancers are associated with infectious agents as major drivers of the pathology. This is likely to be an underestimate as the technological hurdles become more manageable and sensitive in detecting these agents in the cancer tissue; it is likely that this would increase. The discoveries of these associations were supported by strong epidemiological evidence, which has been substantiated by multiple studies. More recently, there were more studies which showed that the contributions of microorganisms do not necessarily have pathogenic consequences but can also be beneficial and in some may provide protective contributions.

The era of the microbiome has given us additional ammunition as to the importance of microorganisms in our daily activities and has shown that homeostasis of our microbial flora is critical to our overall well-being. The large number of investigations into the microbiome at different anatomical sites has demonstrated that the

specific sites of the human body have a preferential microbiome and that changes can lead to the establishment of dysbiosis at these sites resulting in inflammation. This in addition to the direct activities of these agents can function as triggers for proliferation.

This is a complex line of investigation and we now know a great deal more compared to a decade ago. These studies have also provided clear insights into the complex molecular systems, which link microbial homeostasis with inflammation and metabolism, and are based on the physiologic activities between host cells and the microbes that they are associated with in the particular microenvironment. It is also becoming more acceptable due to the plethora of studies to understand the changes in the gut microbiome that different treatment modalities can induce a range of comorbidities in addition to the cancer being targeted. The fact that treatment of cancer patients with chemotherapeutic agents, radiation, and broad-spectrum antibiotics can change the normal microbiota, therefore predisposing the patients to colonization with pathobionts, provided important information as to ways to curb the related comorbidities. Importantly, these changes are likely not only in the gastrointestinal tract but may also affect the microbiota at different anatomical sites. Understanding the changes, which occur, will certainly shed light on potential avenues for interventions.

This initial book is an attempt to address the limited focus on the microbiome associated with the broad range of different cancers along with their microenvironment, and is certainly not comprehensive. I would like to thank the contributors for their time and efforts in attempting to address the more focused area of study related to the microbiome and specific cancers. One major issue we had in assembling this book was that many potential authors were dealing with time constraints and funding so that they were not able to find the time to contribute. Therefore, I am indebted to the ones who found the time from their busy schedule to write the chapters, which are included here. In a time when we are all constrained for time and balancing many other commitments, setting aside the time was a true labor of love. Certainly, there are areas which we have missed due to these constraints, and we hope that in another period, in the future, we would be able to deliver a more comprehensive text as the field becomes more mature. Nonetheless, I believe that the current volume approaches this complex subject area with a wonderful series of chapters. Readers who are novices in the field of microbiome and cancer, as well as more experienced investigators, would find them enlightening. It would certainly be helpful for the many trainees in graduate school or medical school who would like to obtain information that is more concise and focused in this particular area.

As additional studies continue to investigate the cancer-associated microbiome, the differences that will likely exist in the gut microbiota compared to the tumor microbiota will be illuminated. One would expect that there would be some overlap between the gut microbiota and the tumor microbiome in terms of the identified microbiota. However, as more studies related to the tumor microbiome (oncobiome) provide additional data, it will show that, as expected, the volume of microorganisms in the GI tract is much higher than that seen in the tumor microenvironment. Nevertheless, these microorganisms may contribute to the initiation, development,

and maintenance of the tumor microenvironment. They may also be opportunistic, in that the tumor microenvironment would be a perfect place for survival, and may vary based on the oxygen gradient of the tumor, with different levels of hypoxia. The contributions of the entire microbial milieu may also be complimentary. The combined signaling may synergistically drive proliferation and influence survival of the tumor. Clearly, some organisms may have protective influences compared to others, which may be deleterious to the host. This provides a glimpse into the stringent balance that exists in the microenvironment, important for long-term homeostasis.

We have 17 chapters that include the skin microbiome and viruses, microbes associated with glioblastomas, the breast cancer microbiome, ovarian cancer and associated microbiota, the microbiome and lung cancer, infection-induced hepatocellular carcinomas, and manipulation of the host immune system by small DNA tumor viruses. Additionally, we have chapters covering the immune recognition in intestinal cancers, metabolites in promoting and preventing cancer, the virome in hematologic malignancies, esophageal carcinomas and infectious agents, head and neck cancers and infections contributing to its development, mesotheliomas and SV40 infection, and vaccine strategies. Some of these areas are still developing fields, and so we would expect that more information would become available in the near future that would provide greater insights into the role of the oncobiome in cancer.

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Chapter 1
Microbiome and Human Malignancies

Abhik Saha and Erle S. Robertson

Abstract Recent technological advances have revolutionized our current understanding of the role of human microbiota in cancer development. Several high-throughput Next Generation sequencing studies including metagenomics and transcriptomics data, along with microarray-based technologies suggest that dysbiosis in the commensal microbiota can initiate a number of inflammatory syndromes as well as multiple cancers in humans. Immune deregulation by the microbial community is considered one of the major contributing factors for cancer development. In this chapter, we broadly discuss recent developments in understanding the interaction of human microbiome and its contribution to cancer, and the possibilities of future diagnostic, as well as potential for development of targeted therapeutics.

Keywords Microbiota · Cancer · Next-gen sequencing · Metagenomics · Transcriptomics · Microarray

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1.1 Introduction

Cancer is one of the leading causes of morbidity and mortality worldwide, contributing nearly one in every six deaths. As the human lifespan increases, the complexity as well as the incidence of the disease also increases. According to the World Health Organization (WHO) the number of new cases is expected to be amplified by approximately 70% over the next two decades [1]. Out of many established cancer associated factors, microbial infections over the last 100 years have been shown to contribute to nearly 20% of all human cancers, equivalent to close to two million new cases per year [2, 3]. Among the microbial community, viruses are so far the best-studied component for their role in cancer development. These viruses include Epstein-Barr virus (EBV or HHV4), hepatitis B virus (HBV), human papilloma virus (HPV), hepatitis C virus (HCV), human T-cell leukaemia virus (HTLV-1), Kaposi Sarcoma associated herpesvirus (KSHV or HHV8) and the recently discovered Merkel cell polyomavirus (MCPyV) [4, 5]. It is well established that in case of some cancers viral infection appears to be absolutely necessary, such as, HPV infection in the development of anogenital cancers or hepatitis virus (HBV and HCV) infections in hepatocellular carcinoma (HCC) [6, 7]. These have a direct role in driving these cancers as primary contributors. However, it is not yet fully established why some individuals infected with tumor viruses do not develop cancer over their entire lifetime. For example, the majority of the world population (>95%) has been shown to be asymptomatically infected with EBV, the first known human tumor causing virus [[8] and reviewed in [9]]. However, these human tumor viruses drive the development of cancers when the immune system is compromised, exemplified as organ transplant or HIV infected individuals (AIDS patients), and are therefore opportunistic in nature [10].

Although viruses had long been identified as major cancer causing agents, our understanding of the extent of this problem connecting other microbes including bacteria, archaea, fungi and even parasites began only in recent decades and has continued to expand. A growing body of evidence indicates that microbes can play a much larger role in the development of several human malignancies, and indicates the limited understanding of their overall role we have today (reviewed in [11, 12]). For example, recently studies have shown that perturbation of the microbial community (referred to as “dysbiosis”) significantly impairs the response to cancer therapy [12]. Thus, an optimal response to cancer therapy requires an intact commensal microbiota, which regulates the tumor microenvironment through inflammatory cytokines and reactive oxygen species (ROS) production [13].

The microbial kingdom, including bacteria, viruses, archaea, fungi and protists have coevolved with the human system for many years, resulting in intricate host-microbiome interactions and in turn influences a number of physiological pathways—particularly affecting the host immune system [14]. As a result, disruption of the microbiota contributes to a variety of human diseases including immune disorders and cancers (Fig. 1.1) [2, 11, 12, 14]. Cumulative data generated over many decades has enhanced our understanding of the major role that viruses play in devel-

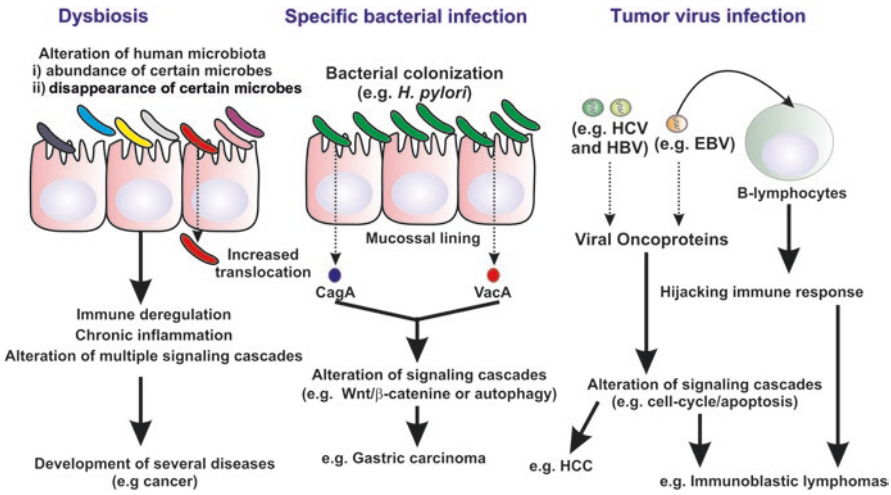

Fig. 1.1 Mechanisms by which microbes promote cancer. Several environmental factors such as diet, cigarette smoking, alcohol consumption, drug treatment and insanitary habits along with genetic predispositions promote 'dysbiosis'—an alteration of physiologic microbiota leading to a number of pathological conditions, including cancer. The alteration of microbiota severely deregulates the host immune response thereby promoting cancer development. Moreover, infections and subsequent colonization of a specific bacterium (e.g. *H. pylori* infection in stomach mucosal epithelial lining) or a virus (e.g. HBV and HCV infections in hepatocytes or EBV infection in B-lymphocytes) through employing their virulence factors, toxins or oncoproteins can also significantly modulate multiple cellular signaling pathways (e.g. *H. pylori* encoded protein CagA activates Wnt/β-catenin signaling whereas VacA blocks autophagy), which in turn lead to development of several human cancers

opment of a number of cancers [5, 15]. While the functions of achaea and fungi in the neoplastic process are largely undefined, a number of recent studies indicated an obvious bacterial association with several human cancers [12]. Of note, *Helicobacter pylori* (*H. pylori*), an early example of an individual member of bacterial community associated with the development of gastric cancer, failed to develop cancer in a germfree mice model [16]. This suggests that *H. pylori* infection alone is not sufficient for cancer development; and that participation of other microbial members appeared to play an important role in the onset of this cancer. On the other hand, in some cases an entire microbial community was shown to promote cancer propagation, such as the transmissible nature of a microbial community in the development of colorectal cancer. In addition, treatment with broad-spectrum antibiotics demonstrated promising outcomes in cancer therapy. Despite recent rapid advances in identifying entire tissue microbiota, delineating the major cancer-causing organism within the microbial community still remains a key challenge in this field. Currently, the field is largely focused on defining the underlying molecular mechanisms governing microbial interactions. A key direction for the field is to identify functional relationships between different microbial kingdoms and the interplay between the tissue specific microbiota (such as, gut microbiome), and multiple cellular pro-

cesses and pathways (such as, the immune system) [17–21]. In this chapter, we will discuss recent development into our current understanding of the overall contribution of different microbial agents in cancer propagation and the opportunity to enhance both diagnosis and therapy.

1.2 Technological Advancement In Lieu of Microbes Associated Cancers

Until recent years, the advent of high-throughput DNA sequencing and microarray technologies have radically changed our perspective regarding the overall infectious/causative agents associated with human cancer development (Table 1.1) (reviewed in [30–32]). These technologies, such as 'Metagenomics', led us to identify the entire microbial pool and the relative abundance of individual members within that milieu (Fig. 1.2). Metagenomics is a powerful tool to understand the human microbiota, describing the diversity of the microbial kingdoms and trans-kingdom interactions [33]. However, metagenomics of a complex biological sample is incapable of revealing gene expression patterns both of host and parasite origin in order to pinpoint functional dysbiosis in the course of development of several human diseases, including cancer. In addition, a significant proportion of the metagenomics data remain un-utilized due to lack of proper reference genomes in the database [34]. For example, more than 80% of the viral DNAs lack reference sequences [35]. Moreover, it is difficult to categorize and maintain the accuracy of the vast amounts of information derived from the moderately short genomic fragments generated by next-generation sequencing, which can result in erroneous annotation. Additionally, the high level of contamination of the human genome is another challenge faced during metagenomics experiments [36]. Nevertheless, through employing metagenomics technology scientists were able to discover Merkel cell polyomavirus (MCPyV), the latest addition in the list of human tumor viruses, in 2008 [4]. A combinatorial approach of various meta-omics including metagenomics, meta-transcriptomics, meta-proteomics and metabolomics can certainly help us understand the precise role of the human microbiome and thereby provide novel strategies for disease management [37]. For example, our group has developed a microarray-based approach (termed as 'PathoChip') containing 60,000 probes for simultaneous detection of both forms of nucleic acids (DNA and RNA) representing all known viruses, 250 helminths, 130 protozoa, 360 fungi and 320 bacteria which are known pathogens. The 'PathoChip' consists of two distinct set of probes—firstly, the 'unique' set of probes for each identified virus, and secondly the 'conserved' set of probes targeting genomic regions that are well conserved between members of a family of viruses, thereby allowing us to detect previously uncharacterized microbial agents. Since the PathoChip technology involves an amplification step, it allows detection of various microorganisms that are present in low genomic copy numbers in tumor samples, or which were fragmented during sample

Table 1.1 Microbiota associated with different cancers[a]

Cancer types	Associated microorganisms	Experiment	Reference
Colorectal cancer	Enriched: Fusobacterium species, Selenomonas, and Leptotrichia species, Enterobacteriaceae, Methanobrevibacter (Archaea, Methanobacteriales), Bacteroides, Roseburia, Ruminococcus, Oscillibacter, Peptostreptococcus, Parvimonas	Metagenomics	[22–24]
Prostate cancer	Enriched: Propionibacterium acnes		[25]
Breast cancer	Enriched: Bacillus, Enterobacteriaceae, Staphylococcus, Comamonadaceae Reduced: Prevotella, Lactococcus, Corynebacterium, Streptococcus, Micrococcus		[26]
Skin cancer	Enriched: Merkel Cell Polyomavirus (MCPyV)		[4]
Acute myelogenic leukemia (AML)	Enriched: Rhizomucor pusillus (zygomycetous fungus)	Microarray	[27]
Triple negative breast cancer (TNBC)	Enriched: Viruses: Herpesviridae, Retroviridae, Parapoxviridae, Polyomaviridae, Papillomaviridae, Bacteria: Arcanobacterium, Brevundimonas, Sphingobacteria, Providencia, Prevotella, Brucella, Escherichia, Actinomyces, Mobiluncus, Propionibacteria, Geobacillus, Rothia, Peptinophilus, and Capnocytophaga Fungus: Pleistophora, Piedra, Fonsecaea, Phialophora and Paecilomyces Parasite: Trichuris, Toxocara, Leishmania, Babesia and Thelazia		[28]
Ovarian cancer	Enriched: Viruses: Yaba Monkey tumor virus, Yaba-like disease virus, Monkeypox virus, Myxoma Virus, human papilloma viruses, herpesviruses Bacteria: Brucella, Chlamydia and Mycoplasma Fungus: Aspergillus, Candida, Rhizomucor, Cladosporium, Acremonium, Alternaria, Cryptococcus, Pneumocystis, Coccidioides, Trichosporon, Malassezia, Rhodotorula, Geotrichum Parasite: Dipylidium, Trichuris, Echinococcus, Strongyloides, Trichinella, Schistosoma, Leishmania, Ascaris, Trichomonas		[29]

[a]The data were derived from various metagenomics and microarray experiments

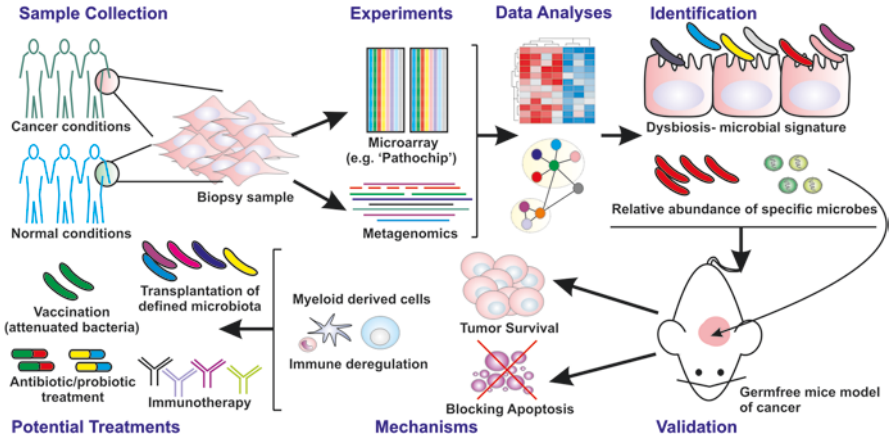

Fig. 1.2 Targeting human microbiota as a potential cancer therapeutic strategy. Through employing high-throughput sequencing (metagenomics) or microarray based technologies it is possible to identify overall microbial composition of disease sample in comparison to normal conditions. Under pathogenic conditions, changes in microbiota composition (dysbiosis) may contribute to cancer development. The 'microbial signature' prevalent in a specific cancer is thus identified and subsequently further investigated in 'germfree' mice model of cancer in order to define the underlying molecular mechanisms. Experiments suggested that microbiota regulates cancer development through blocking immune response and apoptosis, which in turn promotes aberrant cellular proliferation. Treatments targeting microbiota composition, such as antibiotics (to deplete certain bacterial pool), probiotics (to enhance certain microbes), transplantation of defined microbiota (genetically engineered), vaccination using live attenuated bacteria and immunotherapy to regain host immune response have the potential to modulate tumor growth as well as to enhance efficacy of current therapeutic regimen

processing. As a result, this technology has increased sensitivity in comparison to currently available other microbiome screening protocols that involves Next-Gen Sequencing [27]. Furthermore, Next-Gen Sequencing on samples with high microbial load is likely to result in a high degree of selection for the predominant organisms in the sample and therefore selection biases against lower representative organisms. The microarray technology "PathoChIP", although with some limitations in the overall number of organisms, was designed to be inclusive, and so to identify microbial families, which may be represented in the sample [27]. Therefore, this can enrich for organisms that are low representations in the population and thus allow for detection of genomes that are limited in copy numbers. For example, Next-Gen sequencing will have excellent results for acute infections with high copy number of organisms in the gut for example, but may not be as effective for latent infections where few copies of microbial genomes may be present [27]. Using this technology, recently distinct microbial signatures were identified for triple negative breast cancer (TNBC), ovarian carcinoma and oral squamous cell carcinoma [28, 29, 38]. Overall, the identified microbial signatures provide a new paradigm in our current understanding of tumor-associated microbes. However, it is still unclear whether or not these microbes directly contribute to the cancer development or

rather merely exist as commensal microbiota without affecting the cancer microenvironment. Furthermore, the combination of organisms in a population may have additive or synergistic roles in predisposing a tissue to the oncogenic process, or that this combination of organisms has found the perfect niche for their long-term survival. Nevertheless, these microbial signatures provide new diagnostic potential as unique signatures in specific cancers.

To demonstrate the functional importance of the microbiota in cancer development, germfree mice models of cancer were subsequently infected with one or multiple bacteria [39]. However, this 'gnotobiotic model' does not appropriately reproduce the complex composition of the human microbiome. In fact, this experimental approach may either over-emphasize effects due to artificial abundance of a single species or of a group of bacteria, or it may not reveal effects that are due to the requirement of a complex microbial community for the induction of disease by some bacteria. It is therefore imperative to pinpoint the exact environmental conditions that can lead to under-representation and over-representation of certain bacterial species that are associated with cancer, and subsequently to mimic these conditions in experimental models.

1.3 Cancer Associated Microorganisms

To date, the International Agency for Research on Cancer (IARC, http://www.iarc.fr/) categorized 11 infectious microbial agents including seven viruses, three parasites (trematodes), and one bacterium as Group-1 human carcinogens based on their strong association with increasing incidents of several human cancers along with strong evidence from data generated from experiments with laboratory animals (Table 1.2). Although HIV does not directly cause cancer, its infection significantly enhances the occurrence of many tumor viruses (EBV and KSHV) associated human cancers and more recently is also considered an oncovirus, although its effects on the oncogenic process is more indirect. *H. pylori*, HBV, HCV, and HPV together are accountable for more than 90% of all microbes' associated human cancers [56]. The epidemiologic association of some of the human tumor viruses with cancer appeared to be far more complex than what we understood as in general several tumor viruses are highly ubiquitous in nature and found to be associated with more than 95% of the world's population. However, the malignancies that they are associated with are somewhat rare and require specific genetic rearrangements along with number of environmental cofactors that contribute to development of associated cancers. For example, the two gammaherpesviruses—EBV and KSHV are associated with various human neoplasms ranging from epithelial cancers to B-cell lymphomas, particularly in an immune-compromised scenario [57, 58]. EBV is found to be strongly associated with Burkitt's lymphoma (BL), Hodgkin's lymphoma (HL), nasopharyngeal carcinoma (NPC), several form of immunoblastic lymphomas, and to a lesser extent T-and NK-cells lymphoma, gastric and breast carcinomas [9]. KSHV infection causes Kaposi's sarcoma (a rare form of skin

Table 1.2 Group 1 microbial carcinogens[a]

Serial number	Microbial pathogens	Microbial category	Associated cancers	Reference
1	*Helicobacter pylori*	Bacterium	MALT gastric lymphoma, gastric adenocarcinoma	[40, 41]
2	Hepatitis B virus (HBV)	Hepadnavirus	Hepatocellular carcinoma (HCC)	[6, 42]
3	Hepatitis C virus (HCV)	Flavivirus	Hepatocellular carcinoma (HCC)	[6, 43]
4	Human papillomavirus (HPV)	Papillomavirus	Cervical cancer, vaginal cancer, vulva cancer, anal cancer, penile cancer, oropharyngeal carcinoma, head and neck cancer	[7, 44]
5	Epstein-Barr virus (EBV)	Gammaherpesvirus	Nasopharyngeal carcinoma (NPC), Hodgkin's lymphoma (HL), Burkitt's lymphoma, diffuse large B-cell lymphoma (DLBCL) and other immunoblastic lymphomas	[9, 45, 46]
6	Kaposi sarcoma-associated herpesvirus (KSHV)	Gammaherpesvirus	Kaposi's sarcoma (KS), Primary effusion lymphoma (PEL)	[47, 48]
7	Human T-cell lymphotropic virus type 1 (HTLV-1)	Retrovirus	Adult T-cell leukemia/lymphoma (ATL)	[49, 50]
8	Merkel cell polyomavirus (MCPyV)	Polyomavirus	Merkel cell cancer (MCC)	[4, 51]
9	*Schistosoma haematobium*	Trematode	Bladder cancer	[52, 53]
10	*Clonorchis sinensis*	Trematode	Cholangiocarcinoma	[54]
11	*Opisthorchis viverrini*	Trematode	Cholangiocarcinoma	[55]

[a]Designated as per International Agency for Research on Cancer (IARC)

carcinoma) and several other pathologies (such as, Multicentric Castleman's disease or MCD) in immune-suppressive individuals [48]. HBV and HCV are associated with hepatocellular carcinoma (HCC) [59]. HPV, primarily a few high-risk oncogenic strains such as, HPV16 and HPV18, are predominantly associated with several forms of anogenital cancers (cancers of the cervix, anus, penis, vagina and vulva). In addition, HPV is also associated with head and neck cancers, oral cancers and skin cancers [7]. Infection with HTLV-1, the first known human tumor retrovirus, is mostly asymptomatic accountable to approximate 20 million people worldwide. However, in some cases, roughly 3–5% of the infected individuals develop a highly aggressive form of malignancy known as adult T-cell leukemia/lymphoma

(ATL) [60]. MCPyV, the first known human oncogenic polyomavirus, is associated with the majority of cases of Merkel cell carcinoma (MCC), a rare but aggressive form of skin cancer detected in cases of immune-suppressive individuals [[4] and reviewed in [51]].

Interestingly, with the exception of HCV, all human tumor viruses encode at least one oncogene, which was shown to play a direct role in tumor development and progression. However, it has been suggested that many other factors such as inflammation, as well as disruption of the commensal microbiota can also play a role in overall cancer development [13, 61]. For example, even though HPV has a strong transforming ability through exerting E6 and E7 viral oncoproteins mediated activities, vaginal dysbiosis and inflammation around the genital tract due to HPV infection largely contribute to the development of HPV associated anogenital cancers [7]. Hepatitis viruses together with HBV and HCV initially establish a chronic liver infection—a stage known as liver cirrhosis through modulating the host immune response, which eventually develops into HCC, and is accountable to approximately 75% of all clinical observations [6, 59]. The mechanisms by which HBV and HCV promote pathogenesis are distinctly different. Although HBV, but not HCV may directly transform hepatocytes, for both viruses, the pathogenesis of HCC is clearly dependent on immune-related inflammation. While HCV actively evades the initial innate immune response by blocking both type I and type III interferon signaling cascades, the innate immune response to HBV infection is rather weak [62]. However, both viruses are able to compromise the innate as well as adaptive immune responses of the host. Additionally, HBV mediated liver pathogenesis may also connect with gut microbiota particularly the presence of Candida spp., *Saccharomyces cerevisiae,* as well as the less abundance of different varieties of Bifidobacterium spp. Using mice models, the role of the gut microbiota in regulating liver pathology and subsequent development of HCC has been clearly demonstrated as the young mice fail to clear HBV infection until an adult-like gut microbiota is established [63]. It is now clearly understood that inflammation plays a key role in tumor progression associated with all the known tumor viruses. A growing body of evidence clearly suggests that the commensal microbiota along with tumor virus infection are intricately engaged in regulating the immunological response, and thus inflammation which in turn controls cancer propagation, allowing identification of novel molecular targets and their potential for therapeutic interventions [13, 64, 65].

H. pylori infection is considered as the strongest recognized risk factor for the development of gastric adenocarcinoma (non-cardia carcinoma) [66]. Although half of the world's population is infected with *H. pylori*, only a small proportion of individuals develop gastric cancer [67]. In most cases the bacterial infection develops a relatively manageable gastritis, duodenal and stomach ulcers. The worldwide mortality from gastric cancer remains relatively very high, especially in Asia and much of the developing world. *H. pylori* is extremely heterogeneous in nature and is highly adapted for survival in such a hostile condition of gastric mucosa lining contributing a variety of disease pathogenesis. In case of gastric cancer, the major *H. pylori* candidate virulence factors include two cytotoxin encoding genes—cytotoxin-associated gene A (cagA) and vacuolating cytotoxin gene A

(vacA) [68, 69]. Like many other pathogenic and commensal bacteria, *H. pylori* can also profoundly impact the normal functioning of the immune system, of the colonized host. The bacterium activates the TLR4 and TLR2 receptors as well as the NLRP3 inflammasome, thereby promoting the secretion of several interleukins that in turn activate both Th1-cell and regulatory T-cell mediated pro-inflammatory responses [70, 71]. Although *H. pylori* possesses pro-carcinogenic activities and can directly influence gastric mucosa through promoting DNA damage response, development of gastric adenocarcinoma appeared to be much more complicated and involves exposure to the bacterium over several decades, with an initial inflammatory response, epithelium injury and atrophy and a decline in acid secretion function [40, 72–74]. In many developed countries, the occurrence of *H. pylori* infection is decreasing due to better hygiene, recurrent use of broad-spectrum antibiotics and proton pump inhibitors [74]. Interestingly, lowering the incidence of *H. pylori* infection may also result in disruption of the gut microbiota with some unanticipated potential side effects such as, individuals with increased tendency of having asthma, obesity along with elevated risk of development of esophageal and gastric cardiac carcinoma, highlighting the complexity of microbial effects on the development of tissue-specific tumorigenesis [74, 75]. However, this effect may be correlated to a definite genetic predisposition or dietary habits, as the theory was contradicted with the observation, which was found in certain ethnic Malaysian populations known to have a low natural incidence of *H. pylori* infection and generally poor sanitation [76].

With the advent of modern technologies as discussed above, an escalating number of earlier unnoticed pathogens has been discovered, that play critical roles in the development of several human diseases, including cancer. *Fusobacterium nucleatum* (*F. nucleatum*) is such an emerging ubiquitous commensal microbe, usually present in dental plaque, undetected in other parts of the body during normal conditions; however, in disease conditions the bacterium becomes prevalent and disseminates to different body sites. A number of recent studies clearly demonstrated a strong association of *F. nucleatum* with colorectal adenomas and advanced-stage colorectal cancer [23, 77]. For example, *F. nucleatum* introduction to a mouse model of intestinal cancer significantly enhanced the tumor growth through regulation of the NF-κB mediated pro-inflammatory signaling pathway thus affecting the tumor microenvironment [78]. *F. nucleatum* is an adhesive bacterium and encodes several adhesion factors, such as Fap2, RadD, and Aid1 that assist in interspecies interactions in the oral cavity. However, there is only one adhesion molecule, FadA identified, that can bind to the host cells and is one of the best-studied *F. nucleatum* encoded virulence factors [79]. A recent study demonstrated that a host polysaccharide, Gal-Gal-NAc, highly expressed in colorectal carcinoma can be directly recognized by the *F. nucleatum* encoded Fap2 protein, which in turn promotes bacterial attachment [80]. Fap2 also promotes colorectal cancer development by blocking NK-cell mediated immune-surveillance [81]. In addition to the attachment process, FadA can also function as an invasin. FadA inhibits E-cadherin tumor-suppressive activity and consequently, by blocking the interaction of FadA with E-cadherin using a synthetic peptide the host inflammatory response can be abro-

gated, thereby affecting tumor development [79]. Recent studies suggested that *F. nucleatum* increases the ROS production as well as the pro-inflammatory cytokines such as IL-6, IL-8 and TNFα in colorectal cancer [82]. *F. nucleatum* can selectively expand myeloid derived immune cells in colorectal cancer. Myeloid-derived immune cells present in the bone marrow, spleen, or tumor microenvironment can suppress T-cell responses, suggesting a possible mechanism by which *F. nucleatum* modulates the tumor microenvironment and promotes cancer development [83]. In the near future, the detailed elucidation of *F. nucleatum* targeted cellular pathways will provide valuable additional clues for better clinical management of colorectal cancer patients, and their predictive outcomes.

Chronic infections with the liver flukes including *Clonorchis sinensis* (*C. sinensis*), and *Opisthorchis viverrini* (*O. viverrini*) are associated with cholangiocarcinoma [54, 55]. Liver fluke antigens stimulate both inflammatory and hyperplastic changes in the infected bile ducts, which undergo severe pathological transformations. Approximately 5–10% of cholangiocarcinoma is caused by chronic *C. sinensis* infection in endemic areas with low economic status. *Schistosoma haematobium* is a parasitic flatworm associated with bladder cancer that infects millions of people, mostly in the developing world [53]. Research suggest that these helminthes infection are associated with increased cell proliferation, decreased apoptosis, elevation of the anti-apoptotic molecule Bcl-2, down-regulation of the tumor suppressor protein p27, along with increased cell migration and invasion.

1.4 Immune Influence by Microbiota in Promoting Cancer

In recent times, the occurrence of a wide variety of human diseases has been noticeably enhanced, across the globe. The diseases include obesity, asthma, food allergies, inflammatory bowel syndrome, type 1 diabetes and autism, among many others. Ongoing studies have suggested that the disruption and loss of important microbial communities play a major role in the development of such chronic diseases (reviewed in [84]). Loss of such microbial communities have been shown to be associated with changes in living conditions made possible by the introduction of modern life conveniences that has enhanced our daily living standards. For example, extensive use of antibiotics during pregnancy, avoiding breast-feeding and increased rate at which caesarean section is utilized may hamper the horizontal transmission of microbial community from mother to child and in turn result in emergence of several apparently unrelated health problems [84, 85]. An incredible feature of human beings, is not how we respond to pathogenic microorganisms, but more profoundly how we endure the mammoth numbers (estimates of up to three times the total number of host cells) of residing different microbial kingdoms. With the increasing as well as fascinating research in this field, it is now more obvious that the interactions of the early life microbiome with the host are particularly responsible for the commencement of host's immunological tone for the rest of an individual lifespan. Although the most intense effects are focused on the

development of immunity of the gut, microbial communities residing in other areas including skin, mouth and vagina may also contribute to setting the overall immunological, as well as tissue specific immune effects [61, 86, 87].

In addition to emergence of chronic diseases, studies have now clearly demonstrated that microbiota can also influence both cancer propagation and therapeutic response particularly through modulating immune cells and so inflammation. For example, *H. pylori* infection in the gastric mucosa can result in inflammation and aberrant cell proliferation, which subsequently leads to development of stomach cancer [88–90]. On the contrary, a number of intestinal resident bacteria can diminish inflammation, which in turn reduces the rate of cancer cell outgrowth, as well as potentiating the use as cancer immunotherapy. Bifidobacterium can activate dendritic cells in order to present cancer-cell specific antigens to cytotoxic T-cells (CTLs) for killing, which is accompanied by a reduction in growth of subcutaneous melanoma in mice xenograft model. Moreover, introduction of this specific bacterial species in combination with the conventional cancer immunotherapeutic agent "anti-program death-ligand 1 (PD-L1)", can virtually abolish tumor growth [65]. Likewise, combination of *bacteroides thetaiotaomicron* and *B. fragilis* can significantly augment the efficiency of another cancer immunotherapeutic agent 'anti-cytotoxic T-lymphocyte associated protein 4 (CTLA4)' [91]. While *B. fragilis* polysaccharides can enhance anti-tumor immunity, the specific *B. fragilis* polysaccharide A (PSA) promotes an anti-inflammatory state in the intestine [92].

In addition, experiments with pathogen free and antibiotic treated mice demonstrate a typically declined response to CpG oligodeoxynucleotide stimulation in the setting of cancer immunotherapy [13]. The bacterial microbiota also regulates immunity to numerous viral pathogens. It has been demonstrated that previously existing antibodies to enteric bacteria can affect the vaccine responses by cross-reacting with HIV-1 antigens, suggesting a possible mechanistic barrier for proper vaccination [93]. In addition, enteric bacteria can also regulate vaccine responses to influenza in mice through activation of the innate immune receptor, Toll-like receptor 5 (TLR5) [94]. Administration of antibiotics in mice has profound effects on antiviral immunity at another mucosal surface, the lung, since antibiotic treatment prevents normal innate and adaptive immune responses to influenza, causing death of the host [64, 95]. These results emphasize the importance of bacterial microbiota in order to stimulate the antiviral immune responses. However, it is too early for clinicians to decide on using antibiotics as a means of anti-cancer therapy [96]. Expansion of new generation antibiotics targeting individual bacterium along with probiotics [63], as well as introduction of more specific chemotherapeutic agents based on the cancer patient (referred to as 'precision medicine') would definitely change the current scenario (Fig. 1.2).

1.5 Microbes in Cancer Therapy

Owing to the many severe side effects typically associated with conventional chemotherapy, development and inclusion of new anti-cancer therapies are urgently needed. Cumulative studies have resulted in the perception of the microbiota as close associates with their human hosts. Thus, the role of different microorganisms, particularly bacteria and viruses in killing of cancer cells has been explored over extended periods. These studies suggest that these selective microbes should not be harmful to the surrounding non-malignant host cells, and should only replicate in the tumor cells. Furthermore, these microbes should be non-immunogenic and capable of specifically lysing tumor cells [97]. In 1891, an American surgeon William B. Coley observed that administration of certain heat-killed microbes which included *Streptococcus pyogenes* and *Serratia marcescens* (referred to as 'Coley's toxin') can radically cause tumor regression [98]. Therefore, the use of Coley's toxin was often determined as an alternative strategy for the successful treatment of various forms of cancer for which no alternative treatments were available [98]. However, in many cases treatment regimens with Coley's toxin resulted in a number of side effects. This led to limited enthusiasm for this treatment, and is not generally accepted among clinicians. The most promising clinical application of microbial agents in the treatment of cancer was first described in 1976, when a urinary bladder cancer patient was treated by the introduction of the Bacillus Calmette-Guérin (BCG) vaccine, a live attenuated strain of *Mycobacterium bovis, and a* standard vaccine protocol against tuberculosis (TB) infection [99, 100]. Currently, this method is considered as one of the most successful immunotherapy against superficial urinary bladder cancer. In addition, BCG mediated immunotherapy has also been investigated in case of colorectal carcinoma [100]. The anti-cancer effect of BCG is based on the induction of a local immune response and the production of cytokines such as IL-2, TNF-α and INF-γ. However, the BCG vaccine has also shown multiple side effects and incompetence in approximately 50% of the treated patients [100, 101]. Similar to the BCG vaccine, *Lactobacillus* species have also shown promising outcomes in regards to the recurrence of urinary bladder cancer [101]. A number of bacterial species under *Bifidobacterium* genus, including *B. longum*, *B. infantis* and *B. adolescentis* appear to possess potential anti-cancerous agents in mice models [102, 103]. Likewise, several *Clostridium* species such as *C. histolyticum, C. perfringens* and *C. novyi* can also block tumor growth in animal models [104, 105]. Both in case of *Bifidobacterium* and *Clostridium* species, the anti-tumorigenic effects were determined using animal models; lack of patient data and significant associated toxicities raise uncertainties in their therapeutic capacity. Administration of live attenuated *Salmonella enteric* also causes tumor regression in mice models [106]. Subsequently, a genetically modified *Salmonella* strain 'VNP20009' was generated and is being currently tested for the treatment of various cancers in Phase I clinical trial [107]. Later, a number of other strains of

Salmonella species have been generated and demonstrated potential tumor regression activities in various cancer types [108, 109].. Interestingly, natural tumor regression can also occur in the presence of a number of other bacterial infections including Diphtheria, Gonorrhoea, Syphilis and Tuberculosis, and viral pathogenesis such as hepatitis, influenza, rubella and smallpox [110]. In addition, a number of bacterial toxins and metabolites can significantly influence tumor growth both in experimental models and in clinical settings (Table 1.3). For example, while 'azurin', a peptide encoded by *Pseudomonas aeruginosa,* induces apoptosis and

Table 1.3 Microbial agents as anticancer therapy

Anticancer agents	Microorganisms	Mechanism of action	Reference
Azurin	*Pseudomonas aeruginosa*	Deregulates cell proliferation, induces caspase-dependent apoptosis, and blocks angiogenesis	[111]
Exotoxin A	*Pseudomonas aeruginosa*	Inhibits protein synthesis by inducing ADP-ribosylation of cytoplasmic elongation factor 2	[120]
Diphtheria toxin	*Corynebacterium diphtheriae*	Inhibits protein synthesis by inducing ADP-ribosylation of cytoplasmic elongation factor 2, increases apoptosis	[112]
Actinomycin D	*Streptomyces* spp.	Inhibits transcription through blocking RNA polymerase activity	[116]
Bleomycin	*Streptomyces verticillus*	Inhibits DNA synthesis. However, the exact mechanism is not yet known	[114]
Daunomycin	*Streptomyces coeruleorubidus*	Interacts with DNA by intercalation and thereby inhibits macromolecular biosynthesis. It also inhibits the progression of topoisomerase II	[121]
Doxorubicin	*Streptomyces pneuceticus*	Interacts with DNA by intercalation and thereby inhibits macromolecular biosynthesis. It also inhibits the progression of topoisomerase II	[118]
Epirubicin	*Streptomyces pneuceticus*	Forms strong complex with DNA by intercalation between base pairs and also inhibits topoisomerase II activity	[113]
Idarubicin	*Streptomyces pneuceticus*	Forms strong complex with DNA by intercalation between base pairs and also inhibits topoisomerase II activity	[115]
Mitomycin C	*Streptomyces caespitosus*	Inhibits cell proliferation through alkylation of DNA	[117]
Geldanamycin	*Streptomyces hygroscopicus*	Inhibits telomerase assembly through disrupting HSP90-telomerase complex; inhibits src tyrosine kinase activity	[122, 123]
Rapamycin	*Streptomyces hygroscopicus*	Induces autophagy through blocking mTOR pathway	[124]
Wortmannin	*Talaromyces wortmanni*	Blocks autophagy through inhibiting phosphatidylinositol 3 (PI-3) kinase	[125, 126]

blocks angiogenesis, 'endotoxinA' encoded by *Pseudomonas aeruginosa,* and 'diphtheria toxin' encoded by *Corynebacterium diphtheriae* inhibit protein synthesis by inducing ADP-ribosylation of cytoplasmic elongation factor 2 [111, 112]. Interestingly, several species under *Streptomyces* genus produce a number of metabolites (actinomycin D, bleomycin, doxorubicin, epirubicin, idarubicin, and mitomycin C), that act as potential DNA damaging anti-cancer agents at least in laboratory experimental settings [113–118]. However, bacteria and viruses are not the only agents that can induce tumor regression. Additional evidence has shown that a number of protozoa, such as *Toxoplasma gondii* and *Besnoitia jellisoni* can also activate macrophages and thereby causing tumor regression [119]. Although microbial treatment of cancer is providing new perspective, the use of microorganisms to target tumors has certain limitations. For example, the biosafety, genetic instability and the confounding interactions of the microorganisms with chemotherapeutic agents should also be considered in greater detail.

1.6 Conclusion and Future Perspectives

In this chapter, we highlight the recent advances in understanding the human microbiome and its intricate association with cancer, as well as promising future avenues of research, including the identification of novel molecular targets for therapeutic enhancement, development of vaccines and cancer prognostic markers (Fig. 1.2). The host and the microbiome continuously interact with each other, and are considered to be two fundamental constituents of the 'holobiome', resulting in maintenance of a healthy steady state of cellular homeostasis. However, alterations of the host-microbiome interactions coupled with germ-line encoded disease susceptibility risks, resulted in onset of several disorders, including cancer. The advent of high-throughput technologies has radically changed our understanding of the host microbiome and its ability to play a major role in cancer development. However, extensive research will be necessary to delineate the roles of organ-specific microbiome in cancer development. The effects of one microbiome on tumor progression in other distal locations, and alterations in immune functions by the microbiota, as well as the potential involvement of other commensal microbial kingdoms, such as fungi, archaea and parasites, along with environmental factors (such as food habit, smoking) in cancer biology needs to be further explored. As the scientific community continues to generate more microbiome data, and integrate other "omics" types such as transcriptomics, proteomics, and metabolomics from well-phenotyped cohorts, we would be able to discover novel microbial signatures that are associated with disease onset and progression in many diseases, including cancer. These microbiome signatures along with circulating metabolites have the potential to be utilized in diagnostics and therapeutics strategies.

Overall, the outlook is optimistic, but there are also substantial challenges in the field. To implement microbiome-based diagnostics and therapeutics, we need to develop uniform collection, sequencing, and analysis standards that would

enhance reproducibility of results across centers and reduce biases in their interpretation. In general, the recent investigations are based on identification of microbes associated with different cancers. However, the trend should be towards better defining the underlying mechanisms by which microbiota manipulate cancer microenvironment along with development of appropriate biomarkers. Once the most favourable microbial composition for each clinical condition has been identified, the next challenge will be how to modify the patient's microbiota in order to enhance cancer therapy. In addition, we are only beginning to appreciate the contribution of other microbial kingdoms such as fungi, bacteriophages, and parasites as well as the transkingdom interactions along with host cellular signalling pathways. As we unravel aspects of these intricate interactions, we will begin to understand the influence of the microbiome with both positive as well as negative regulatory impacts, on the host in connection with development of various pathophysiological conditions, such as cancer. Although the field of therapeutic intervention through targeting the microbiota is still in its infancy, a number of approaches has already been made. For example, the validation of the microbiota as a therapeutic target is provided by studies showing that patients can be recolonized with a resilient and stable modified microbiota to fight antibiotic resistant pathogens. The ultimate goal is to discover a bacterial species or a combination of species that both reduces systemic toxicity and promotes anticancer therapy. Thus, targeting the microbiota in cancer and other diseases is likely to become one of the next frontiers for precision and personalized medicine.

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