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Microbiota: A Double-Edged Sword in Cancer Therapy

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Abstract: ***The relationship between the host and its microbiota can be symbiotic or pathogenic depending upon the body's immunity standards. Experiments have demonstrated that germ-free or antibiotics treated mice showed increased colorectal and pancreatic cancer risk. This shed light on the central role of microbiota in inflammation and immune system regulation of the host; both get impaired during cancer development. Microbial dysbiosis depending on the host's genotype, antibiotics exposure, diet alteration, and cancer therapy can either promote or suppress the diseased state. Thus, the host and its microbiota association is being dissected thoroughly and there exists a possibility in using microbiota as a target or a weapon to kill tumors. Cancer treatment remains a challenge to date due to the heterogeneity of solid tumors concerning the genetic make-up, histopathological features, and clinical behaviors. Bacteria can be used as gene/drug delivery vehicles (weapon) to target tumors. Besides, intratumoral bacteria can also be targeted to indirectly inhibit cancer growth. This is because microbial signals induce anti-tumor immunity via CD8+ T cell activation, prime myeloid cells for TNF production in response to CpG oligonucleotides, produce ROS in response to platinum salts (oxaliplatin), and regulate the efficacy of anti-CTLA-4 and anti-PD-L1 therapy. Therefore, in combination with these conventional cancer therapies (chemotherapy and immunotherapy), consideration of microbial consortium of the patient is a novel approach and has helped increase the efficacy of anti-tumor therapies.Here, in this review, the aim is to elucidate the role played by microbiota in cancer development and how it can be manipulated for tumor targeting, and enhancing cancer therapy. Also discussed are other clinical regimens like probiotics and fecal microbiota transplantation wherein the role of microbiota is central.***

Keyword: Microbiota, Immunity, Cancer, Inflammation, Microbial dysbiosis, Cancer therapy, Bacteriotherapy, Probiotics, Fecal microbiota transplantation

1. Introduction

Microbiota is the medley of microorganisms present in a defined environment while collection of genes of the members constitute the metagenome. Microbiome is another important term referring to the entire habitat including the residing microorganisms (bacteria, archaea, viruses, and fungi), their genomes, and the surrounding environmental conditions. All the metabolites in any given tissue or strain is called the metabolome [1]. Hence, along with its microbiota it is not incorrect to refer humans as 'meta-organisms' [2].Ever since the life began, hosts have evolved around microbes and gradually with the passage of time, communication between the two via biologically active molecules and metabolites led to regulation of important physiological aspects of the host such as nutrition, metabolism, immunity, inflammation and neurological functions [3]. It has been established that microbes roughly exceed the human somatic and germ cells ten times and inhabit the epithelial barrier surfaces of numerous human body sites like skin, mouth, nose, gut, uterus, and so on [4]. Of these, the largest surface area of interaction is comprised of the gastrointestinal (GI) tract; inhabited by the commensal microbes referred to as the 'gut microbiota' [5]. Even in the GI tract, gradients of pH or oxygen cause stratification of distinct microbial communities in different regions namely colon and small intestine [6].

Since, the relationship with microbes can be either commensal, mutualistic or pathogenic, homeostasis has to be maintained between symbiosis and pathogenesis [7]. Various factors like diet, antibiotics, lifestyle, alcohol intake, stress, chemotherapy, and anti-viral drugs are known to perturb the host-microbiota interaction [8]. As a consequence of this disturbance in the resident microflora which is called 'microbial dysbiosis', many pathological conditions arise such as type II diabetes, inflammatory bowel disease (IBD), coeliac disease, and different cancer types (colorectal, pancreatic, gastric, esophageal and hepatocellular cancer) [9].Cancer, a major public health problem, is a multifactorial pathology and has become the second leading cause of death worldwide [10]. Over the past few years, various studies have uncovered the role of microbiota as a double-edged sword in regulating the host's health and disease, particularly in cancer. Any imbalance in the mechanisms of regulating immunity and inflammation might shift the activity of microbes from being tumoricidal to tumorigenic. This is because some microbes are protective against tumor genesis while on the contrary, during dysbiosis, some subpopulations of microbiota that expand and produce high levels of toxins have the potential to trigger inflammation and tumorigenesis [5]. A very common example is colorectal cancer (CRC) where species like *Fusobacteriumnucleatum* induce cancer risk via the production of toxins and activation of pro-inflammatory pathways while *Bifidobacterium spp.* play a role in protection against tumors [11]. Similarly, dysbiosis of specific bacterial species in different cancer types has been worked out and has revealed the potential in microbiota modulation to venture tools for treatment purposes. Also, various microbial biomarkers for cancer are being elucidated for diagnostic and treatment purposes.

Having known the involvement of microbiota in the host's biological functions and diseased state, it is evident that the immune response to and the efficacy of traditional cancer

therapies like immunotherapy, radiotherapy, and chemotherapy is also regulated by the commensals [12]. This happens due to the central position occupied by microbes in interacting with both immune and tumor cells [13]. Henceforth, for designing better treatment options of cancer, an open-minded approach is required combining conventional cancer therapies with microbiota modulation; which along with a reduced inflammatory response will also increase the efficacy of the therapy [14]. Examples of these include fecal microbiota transplant from a healthy donor, use of probiotics, genetic manipulation of bacteria involved in elevating cancer risk to reduce toxicity, and change in dietary habits to indirectly colonize the gut with healthy bacteria. These therapeutic regimens will be a breakthrough in the race for being disease-free. Furthermore, metagenomics studies can help correlate particular populations, genera, and species with disease incidence.

2. Literature Review

2.1 Gut-Brain- Microbiota: Crosstalk

The crosstalk between the intestinal microbiota and the brain is held responsible for regulating the host's metabolism, immune system, and protection against pathogens [5]. This is because of the constant functioning of a bidirectional loop of the gut-brain-microbiota axis (GBMAx); depicted in figure 1 [15].

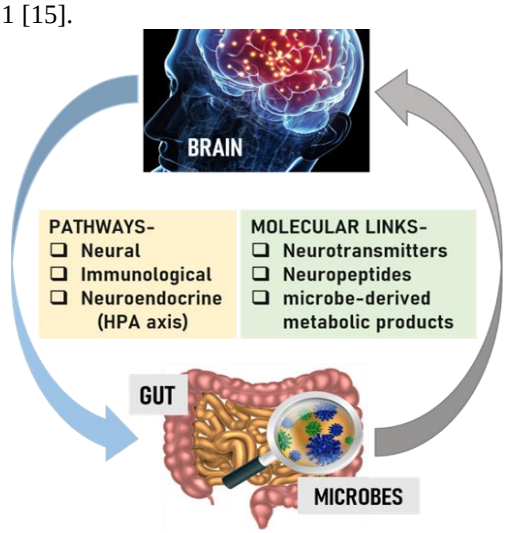

Figure 1: Gut Brain Microbiota Axis

From Figure 1, GBMAx is a bidirectional loop that functions reciprocally between the gut and the brain. Regulated by each other, they communicate along different pathways (yellow) viz. neural, immunological, and neuro-endocrine. Gut bacteria sense the hormones released by the entero-endocrine cells, and secrete biologically active products whose effects are transduced to the GBMAx. In response, the overall gut microbial composition is determined by the neurotransmitters and neuropeptides {molecular links (green)} released by the brain [15]. Microbial products might physiologically link the gut to other organs; implicated in metabolic diseases [5].

2.2 Gut biogeography- Stratification along different gradients

Specific microbiota populating different host habitats during different life stages is major in anticipating the role it plays in the host. Figure 2 depicts the compartmentalization of bacteria in the GI tract that ensures the bacteria do not invade, and injure the host's underlying tissues and help sustain homeostasis [6]. Early in life, the overall microbial diversity is low. Progressively with age and exposure to environmental factors, diversity expands and an adult comprises a diverse but distinct/host-specific microbial profile. *Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, and Verrucomicrobia* are the commanding bacterial phyla in the gut, out of which 90% is constituted by *Firmicutes* and *Bacteroides* [16].

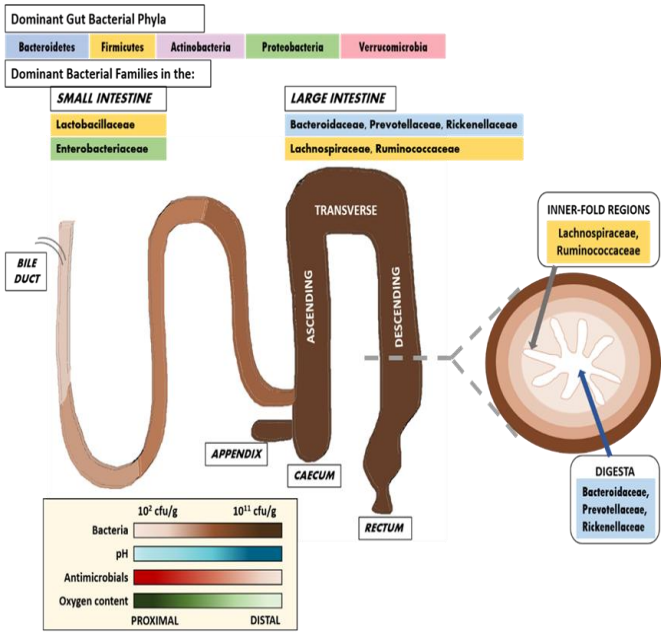

Figure 2: Gut biogeography- Stratification along different gradients

Figure 2 shows, Gut biogeography depicting stratification of bacteria along the longitudinal (proximal and distal regions) and transverse (lumen to mucosa) axes of the GI tract. It exists due to the gradients of physical (the epithelial and mucus layers), biochemical (enzymes and anti-microbial peptides, AMPs), immunological (immunoglobulin A secreted by epithelial cells of the colon and SI), pH, and oxygen level factors. Because of the reduced gradient, in the colon, bacterial families like *Bacteroidaceae, Prevotellaceae, Rikenellaceae, Lachnospiraceae* and *Ruminococcaceae* dominate. Higher concentration of these factors in the SI allows the fast-growing facultative anaerobes of bacterial phyla like *Firmicutes* and *Proteobacteria* to dominate. Along the length of SI as the gradient of host-derived bile acids and AMPs decreases towards the distal end, the overall bacterial diversity in the distal regions increases. The transverse section of the colon shows that the lumen (digesta) has abundant *Bacteroidetesspp.* than the mucosa (inner fold region) where *Firmicutes* are more. Further, the outer mucus layer is colonized by *Bacteroidesacidifaciens*, *Bacteroidesfragilis*, and *Akkermansiamuciniphila* while the inner layer is

inhabited by *Bacteroidesfragilis* and *Acinetobacter*spp. [6]. (Families belonging to a particular phyla are shown with the same color. Gradients are depicted using distinct colors, with shade and tint reflecting the highest and lowest concentrations, respectively)

2.3 Effect of microbes on the host

Microbes can be fungi, bacteria, or viruses. Here, the focus will be on the contribution made by bacteria in affecting the host's metabolism, resistance against pathogens, and immunity [17].

2.4 Benefits to the host metabolism

Experimental studies on rodents and humans have shown that any disturbance in microbiota is implicated in the form of metabolic diseases like IBD, type II diabetes, coeliac disease, and cancer [17]. Some examples where microbiota assists in the host's metabolic processes are listed here.

1) In the gut, *Bacteroidesfragilis, Enterobacteragglomerans,* and *Enterococcus faecium* anaerobically synthesize menaquinone i.e. vitamin K2 and lower the risk of cardiovascular disorders because of decreased vascular calcification, and hiked HDL levels [18].
2) Only intestinal microbiota can synthesize vitamins B5 and B12 which function as coenzymes in biochemical metabolic pathways [18].
3) Certainbacteria can modify small amino acids into signaling molecules; like histidine to histamine or glutamate to gamma-aminobutyric acid (GABA) [5].
4) *Bacteroidetes* and *Firmicutes spp.* ferment dietary fibers in the colon to produce succinate and short-chain fatty acids (SCFAs) like butyrate that travel through the bloodstream to control lipid and glucose metabolism by activation of intestinal gluconeogenesis [5]. *Prevotellacopri* when given to the mice by gavage in the presence of dietary fiber, showed improved glucose control because of succinate production [19]. These functions are regulated by the G-protein coupled receptor (GPCR) signaling [18].
5) Abundance of *Akkermansiamuciniphila*, in the mucus layer, decreases in obese mice but upon fiber feeding the number restores to normal because it alters glucose levels and improves gut barrier by reducing circulating inflammatory lipopolysaccharides [20].

2.5 Colonization resistance against pathogens

Rolf Freter hypothesized, whether or not a bacterial species can utilize a specific limiting nutrient determines its ability to colonize the gut [21]. In recent years, experimental evidences have proved that substrate competition bespeaks about the indigenous abundance of each species [6]. Resident microflora generate substrates that may be utilized by opportunistic harmful bacteria. So, the residential microbiota plays a protective role against harmful bacteria by competing for nutrients and sites of colonization. This is called as colonization resistance; resident microbes being better in utilizing specific limiting nutrients [7].

2.6 Players in Host Immunity and Inflammation

The Host immune system evolved in association with diverse microbiota and their interaction determines the host's immunity standards [22]. Studies have shown that germ free mice or the ones treated with antibiotics had their immune system impaired [23]. Hence, microbiota possesses the ability to regulate the development and function of the host's immune system as well as infection. Mother's gut, vagina, and breast milk expose the baby's immune system to her commensals; thereafter exposed to environmental factors. Mode of delivery has immense effects on neonatal immunity. Epidemiological studies showed that infants born after cesarean delivery were more prone to atopic diseases and allergic reactions, and their skin flora constituted the pathogenic bacteria found on skin and hospitals, such as Staphylococcus and Actinobacter whereas babies born via vaginal delivery had abundant Lactobacillus genera which promoted cytokine production, implicated in neonatal immunity. Further during development, breast milk rich in IgA, live microbes, metabolites, immune cells, and cytokines is responsible for shaping the microbiota of breast-fed infant and immune response to these microbes [24]. Interestingly, the role of commensals in the development of secondary lymphoid structures has also been revealed experimentally. Germ-free mice have reduced Peyer's patch size and IgA- secreting B cells and CD4+ T cells [22]. Above information conclusively proves that the primary encounter of the host immune system with microbial components is important in establishing immune system homeostasis.

Antigens in the host can be self, environmental, diet, or microbiota-derived. As a fact, most of the immune cells reside at the sites occupied by microbes like skin or GI tract and process local signals like metabolites, hormones, and cytokines to generate a homeostatic response. To achieve homeostasis with limited inflammation of the host's tissues and microbial translocation, microbes should be constrained within defined regions. In the GI tract, the gut barrier formed by the mucus layer and epithelial cells (generate AMPs) avoids microbial infiltration into the underlying tissues [18]. The relationship is complex as the If immune response against these antigens get misfired then different pathologies such as allergies, metabolic syndromes, autoimmunity, and inflammation result [23]. The mechanisms by which bacteria calibrate the function and response of the immune system in a normal individual are explained:

1) Commensal microbial antigens that cross the epithelial barrier are presented by the dendritic cells (DCs) and lead to differentiation of commensal specific T-regulatory (Treg) cells, Th17 cells and IgA secreting B cells. Gut residential DCs produce retinoic acid and TGFβ involved in Treg cell induction [23]. Bacteroidesfragilis produces Polysaccharide A (PSA) which engages with TLR2 and ends up inducing Treg cells along with the suppression of TH17 effector response. Clostridium sp. creates TGFβ in abundance and also stimulate Treg cells function. Intestinal bacteria break dietary fibers to produce SCFAs that regulate Treg network by modulating gene expression due to inhibited HDAC (Histone Deacetylase) activity.

The differentiated Treg cells maintain tolerance for commensal microbes. Overall, the aided regulatory function and suppressed effector response prevents inflammation [22].

2) When whole bacteria translocate through the barrier then they are engulfed by macrophages for rapid IL-1b activation in lamina propria or carried away by DCs to lymph nodes.Invasion of pathogenic species is dealt by the body's protective inflammatory response during which macrophages, DCs, neutrophils, and NK cells produce reactive oxygen species (ROS) resulting in increased levels of cyclo-oxygenase 2 (COX-2) and epithelial cells' DNA damage. During acute mucosal infection, inflammatory monocytes respond to microbial agents by producing prostaglandin like mediators (PGE2) which in turn limit neutrophil activation and thus tissue damage [22].

In addition to the myeloid cells, inflammasomes, present as large multiunit complexes on the immune and epithelial cells, recognize metabolites from commensal or pathogenic bacteria using nod-like receptors (NLRs) and maintain tissue integrity and immune homeostasis. In cancer, the role of inflammasomes can either be suppressing or promoting depending upon the effector activated. It has been studied that IL-18 activation results in CRC suppression but IL-1β activation causes pro-inflammatory action resulting in tumor promotion in tissues like lung, skin, breast, and pancreas [5].To sum up, the microbiota educates and instructs the immune system' development, function, and regulation. In disease advancement, the action of microbes relies on the host's immune activation, its genotype, and the localization of microbes [22]. The immune regulatory pathways help in the continuity of mutual terms with microbiota and the systemic control of inflammation withal. Breakdown of any regulatory network can lead to different disease types, especially cancer [5].

3. Methodology

3.1 Microbial dysbiosis and Cancer

In 1984 it was observed that ulcers and cancers pertaining to stomach had a single bacterial type as the causative agent i.e. Helicobacter pylori; characterized as a class I carcinogen by the World Health Organization (WHO) [9]. The corollary of this was the role played by commensal microbiota in cancer development. The fact that most of the people carrying this particular bacteria do not exhibit symptoms and that it also protects from other cancer types, stresses the point that commensals aren't always causing pathological conditions [22]. Some microbes also help suppress tumor. Considering the genuine role of microbiota in inflammation and immune function regulation, whether or not inflammation and tumorigenesis is induced depends on the host's response to the colonizing microbes. And, now the emphasis has shifted to the effect microbial dysbiosis (not just one species) has on cancer initiation and progression [16].

Post-birth, such multiple factors as diet alteration, antibiotic exposure, illness, lifestyle practices, stress, anti-viral drugs, and chemotherapy seed the foundation for rapid shift (reduction and/or changeover) in the diversity of microbes colonizing the GI tract (Figure 3). This shift is termed as 'microbial dysbiosis' and can enmesh the host in multiple inflammatory and autoimmune states; in particular, inflammatory bowel disease (IBD), multiple sclerosis (MS), type I diabetes, and rheumatoid arthritis (RA). This alteration is also implicated in the etiology of colon, pancreatic, gastric, esophageal, breast, laryngeal, liver, and gallbladder carcinomas. Dysbiosis results in increased cell proliferation by providing a microenvironment that alters stem cell dynamics, and the production of metabolites that affect immune response and metabolism; in turn increases the risk for cancer [9]. A study highlighted the correlation of antibiotic exposure with microbial dysbiosis in 15 different cancer types. They observed that cancer risk increased with increasing number of antibiotic courses prescribed (> 10) and especially penicillin use for more than 1 year. Also, when mice genetically susceptible to CRC were given high-fat diet, a distinct microbiota composition with a causative role in tumor progression was observed; this phenotype could be transmitted to healthy mice using fecal samples. Hence, they concluded that antibiotic exposure can alter the microbial diversity in different body sites; dysbiosis was described in cancer patients, the type and dose of certain antibiotics increased cancer risk [25].

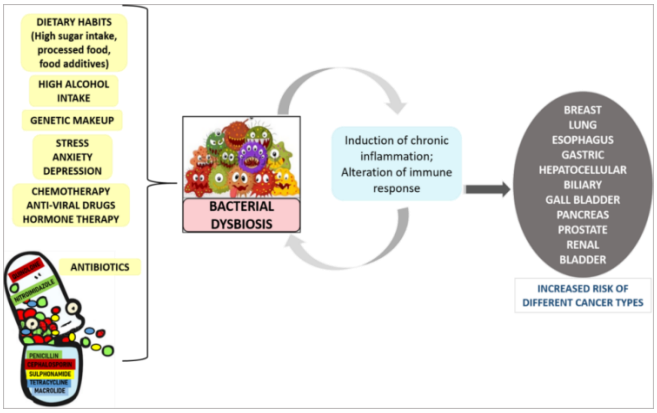

Figure 3: Microbial dysbiosis and Cancer

Figure 3 illustrates, Multiple factors are responsible for perturbing the microbiome content; in turn increasing predisposition to cancer. This etiology is linked closely to the host inflammation that causes and is provoked by microbial dysbiosis [16], [25].Understanding these aspects gain importance when giving cancer therapy because patients have distinct genotype and microbiota, with the potential to alter the response to these therapies. Further, discussed is the role of microbial dysbiosis in causing different cancer types and how the immune cells respond to this alteration.

3.2 Effect of Microbial Dysbiosis on Etiology of Different Cancer Types

3.2.1 Colorectal Cancer

CRC, the third most common cancer, has multiple genes and factors as the underlying cause. The role of dysbiosis in the same is being understood these days with the advent of sequencing and high scale data analysis. A group of researchers, showed the occupation of colon tumor sites by specific bacterial species like Fusobacteriumnucleatum (F.

nucleatum), by metagenomic sequencing of fecal samples [16]. Further studies identified that Escherichia coli (E. coli), Bacteroidesfragilis (B. fragilis), and Enterococcus faecalis (E. faecalis) sufficiently increased in abundance in CRC patients. On the other hand Clostridiales, Faecalibacterium, Blautia, and Bifidobacterium were absent [17]. Different bacteria manifest different mechanisms to affect CRC risk and are highlighted in Table 1. For example, F. nucleatum, implicated in colon adenomas and cancer, induces cancer genesis through the inflammatory nuclear factor-kappa b (NF-kb) signaling pathway and also by down-regulation of anti-tumor T cell-mediated adaptive immunity [9]. In addition, FadAadhesin that binds E-cadherin mediates invasion of F. nucleatum into the epithelial cells which activates pro-inflammatory and oncogenic signals [16]. In total, accumulating evidence points toward the participation of not just one bacterial type but a cluster of them in CRC development [11].

Microorganism	Gram +/-	Expression/ role in CRC	Mechanisms used to induce/suppress CRC risk
E. faecalis	Positive	Induction	Production of super oxides causing DNA breaks
Shigella	Negative	Induction	Inflammation induction
E. coli	Negative	High	Production of cyclomodulins that are either genotoxic or interfere with cell cycle; colibactin damaging DNA; induction of epithelial proliferation
B. fragilis	Negative	Induction	Pro-inflammatory B. fragilis toxin (BFT) production, involved in NF-kB, Wnt and mitogen-activated protein kinase (MAPK) like signal transduction pathways inducing Th17/IL-17 inflammatory response
Streptococcus bovis	Positive	High	Chronic inflammatory response
H. pylori	Negative	High	Multi-function toxin VacA produced
F. nucleatum	Negative	High	Triggers inflammatory nuclear factor-kappa b (NF-kb) and Wnt signaling pathway; reactive oxygen species (ROS) increased; highly enriched in CRC
Bifidobacterium	Positive	Protection	Reduces β-glucuronidase activity in CRC cells; enhance chemotherapeutic efficacy
Eubacterium rectale	Positive	Low	Butyrate producer
Clostridium septicum	Positive	Low	Secondary bile acid producer
Faecalibacterium prausnitzii	Positive	Low	Butyrate generation
Lactobacillus	Positive	Protection	Lactic acid production reduced; activation of TLRs; induced expression of p53 and BAX leading to apoptosis and inhibition of inflammation

Table 1: Bacteria specific to colorectal cancer (CRC) [11]

3.2.2 Pancreatic Cancer

Various follow-up studies were carried out in different continents to know about the periodontitis and pancreatic cancer (PC) association. Periodontal disease is induced by dental plaque bacteria and results in inflammation of tissues that surround teeth. All observations showed increased PC risk in individuals with a history of periodontal disease. Numerous epidemiological studies helped explore the role of oral bacterial perturbation in pancreatic cancer. Among the keystone oral pathogens involved were Neisseria elongate, Streptococcus mitis, Porphyromonasgingivalis, and Fusobacterium [26].

Even gut bacteria were found to be associated with PC risk factors like obesity and type II diabetes. Another study showed that human pancreas contains a microbiota that is unable to contrast normal tissue and an adenocarcinoma; the capability to differentiate is endowed by the gut microbiota (non-pancreatic) that reach pancreas either through the biliary/pancreatic duct or the circulatory system [27]. Table 2 depicts the association of oral, gut, and intratumoral bacteria with PC risk. Further, Fusobacteriumnucleatum and Granulicatellaadiacens were the most abundant at tumor sites. These specific bacteria at distinct sites are seen as potential biomarkers for PC detection. Leptotrichia, Pseudoxanthomonas, Streptomyces and Bacillus clausii were shown to have protective abilities against tumors via immune system modulation [28]. Also, viruses like Hepatitis B virus and Hepatitis C virus can reach extrahepatic tissues like the pancreas and cause carcinogenesis by replicating and integrating in the cells [26].

Microorganism	Sample type	Gram +/-	PC Risk	Mechanism
Neisseria elongate	Salivary	Negative	Decreased	-
Streptococcus mitis	Salivary	Positive	Decreased	-
Bacteroides genus	Oral wash	Negative	Increased	-
Firmicutes phylum	Salivary	Positive	Increased	-
Porphyromonas gingivalis	Oral wash & blood antibody	Negative	Increased	Produce peptidyl-arginine deiminase (PAD) capable of degrading arginine & may result in p53 and K-ras mutations; initiate inflammation
Aggregatibacter actinomycetemcomitans	Oral wash	Negative	Increased	-
Fusobacterium	Salivary	Negative	In one study decreased while in other increased	Might increase (ROS) production & inflammatory cytokines; tumor immune microenvironment modulation; infiltration of myeloid cells in intestinal tumors.
Leptotrichia	Salivary		Decreased	Protective role in a dose-dependent manner
Bacteroidetes phylum	Stool	Negative	Increased	-
Firmicutes phylum	Stool	Positive	Decreased	-
Actinobacteria phylum	Stool	Positive	Decreased	-
Helicobacter pylori	Serum (IgG antibodies against H. pylori)	Negative	No association till date.	If associated then indirectly via inflammation and immune escape
Hepatitis B virus (HBV)/ Hepatitis C virus (HCV)	Viral DNA load			Inflammation induced; tissue viscoelasticity modified, infected cells integrated with DNA and delays clearance of infected cells; PI3K/AKT pathway modified

Table 2: Oral, gut and intra-tumoral bacteria specific to Pancreatic Cancer [26], [28]

3.2.3 Gastric and Esophageal Cancer

In stomach, *H. pylori* promotes gastric cancer by increasing cell proliferation that elevates gastric mucosa turnover and mutation rates so that less DNA repair time is available. This is because the product of cytotoxin associated gene A (CagA) of *H. pylori* activates proteasomal degradation of p53 in epithelial cells; AKT and MAPK pro-survival pathways are involved [5].In the esophagus the role of *H. pylori* is less pronounced. But at the junction formed by the esophagus and gastric cardia, tumors are associated with *H. pylori*. In a study, tissue from esophageal cancer showed a higher abundance of *Treponemadenticola*, *Streptococcus mitis*, and *Streptococcusanginosus*; all produce cytokines to induce inflammation and trigger tumorigenesis [17].

3.2.4 Hepatocellular Carcinoma

The liver is in contact with bacterial components from the intestine via the portal venous system. Microbes convert primary bile acids to secondary bile acids like deoxycholic acid (DCA) that damage DNA and induce hepatotoxicity and carcinogenesis. Increased *E. coli* has been reported in hepatocellular carcinoma.

3.2.5 Breast Cancer

16α estrogen hydroxylation is catalyzed by intestinal microflora and altered estrogen (steroid) metabolism is correlated with increased risk for breast cancer development [9], [17].

3.2.6 Laryngeal Cancer

Bacteria like *H. pylori*, *Fusobacterium*, *Prevotella*, and *Gemella*and human papillomavirus (HPV), form biofilms to stimulate inflammatory response to cause laryngeal tissue carcinoma [17].In most of the cancers described, the dominance of bacteria shifts from gram- positive to negative known to survive anoxic tumor conditions. Therefore, it can be concluded that microbial dysbiosis kicks the tumorous state. Suffice it to say that in a balanced condition, microbiome bolsters human health but if in a havoc due to inflammatory events and many reasons, the upshot is dysbiosis with heightened vulnerability to pathogens. Presuming the successful establishment of these pathogens, chronic inflammation results in continuity with the impaired microbiota. Further, microbes deploy diverse mechanisms to potentially raise cancer risk

3.3 Overall mechanisms the microbes exploit to induce tumorigenesis

Examples from various cancer have proved that a multitude of mechanisms are utilized by the microbes to aggravate different kinds of cancer. The central mechanisms are explained-

3.3.1 Bacterial metabolites can induce local and systemic effects, affect gene expression and inflammation

1) Prostaglandin E2- elevates inflammation and therefore cancer risk.
2) Short Chain Fatty Acids (SCFA) are ligands for GPCRs on the host cell. Acetate is utilized by lung, breast, and ovarian cancer cells for growth.
3) Bile salts in the liver are deconjugated by anaerobic bacteria like Bacteroides, Eubacterium, and Clostridium spp. Intestinal microbes like Lactobacillus and Escherichia metabolize deconjugated primary bile acid to produce secondary bile acids (SBA) that induce tumorigenesis utilizing the MAPK pathway. Examples of SBA are deoxycholic (DCA), a ligand for farnesoid X receptor (FXR), or Vitamin D receptors which themselves are transcription factors; and lithocholic acid (LCA), produced by B. fragilis to prevent DNA damage [3].

3.3.2 Direct effects on signal transduction pathways

Microbes when invade host cells, promote cell longevity by delaying host cell turnover. This could be achieved by apoptosis inhibition due to effects on p53 or up-regulation of cell cyclins [3].

3.3.3 Modulation of the host's physiology and response

This can be achieved by disturbing hormone metabolism and inflammation.

(a) Hormonal perturbations

It has been found that breast cancer patients who were treated with ampicillin showed increased estrogen metabolites in feces because the antibiotic treatment had disturbed the intestinal flora and lowered re-absorption of estrogen [17].

(b) Inflammation Induction

Microbes when infiltrating through the barrier, result in chronic inflammation by toll- like receptor (TLR) signaling. TLRs are pattern recognition receptors and most of them by using MyD88 as the adaptor, affect downstream effectors like NF-kB, MAPK, and interferon regulatory factors. Different bacteria exploit different TLRs for inflammation induction. Increased *Fusobacterium*induces TLR 4 expression (ligand is lipopolysaccharide) to activate NF-kB implicated in tumor growth. Bacterium flagellin is recognized by TLR 5 that leads to the activation of NF-kB plus inflammatory cytokines like IL-17/ IL-22. TLR 2 perceives *Bacteroidesfragilis*produced polysaccharide A and results in the inhibition of Foxp3+ Treg cells and promotion of T helper 17 cells' effector response that induces inflammation [16].

Immune system cells such as B cells, NK cells, and monocytes express TLR 9 with the ability to bind unmethylated CpG sequences in bacterial DNA. Evidence shows that cancer cells with higher genotoxic stress have TLR 9 level hyped significantly [14].As a consequence, chronic inflammation may promote neoplasia by increasing the probability of genotoxic effects in addition to the mutagenic events.

3.3.4 Effects on other microorganisms

Microbes present in a tissue affect the functioning of other microbes. This can be achieved by any of the above-mentioned processes. For instance, H. pylori might allow other microbes with genotoxic abilities to trespass host cells and promote pathophysiologic changes for a much longer time period [3].

3.4 Tumoricidal effect of microbes

While some bacteria induce tumorigenesis, others localize to and interact with the tumor microenvironment, and alter the immune cells, cytokines, and chemokines that infiltrate to alleviate tumor growth. Several bacteria have intrinsic tumoricidal effects and a few of them activate specific immune cell populations against tumor cells [29]. Table 3 summarizes these bacteria involved in tumor suppression.

Microorganism	Biological effector	Mechanism
S. Typhimurium, Listeria	Toxins	Induce apoptosis or autophagy, accumulate granulocytes and CTLs
Flagellated bacteria	Bacterial flagellin	Via TLR 5 activation, anti-tumor response of CD8+ T cells is enhanced
E. coli	Cytolysin A (ClyA)	Pore forming hemolytic protein; cytotoxic agent
Clostridium spp.	Hemolysin, Phospholipases	Recruit neutrophils at the tumor site; high secretion of TNF-α and TNF-related apoptosis-inducing ligand (TRAIL) enhances immune response; tumor elimination by apoptosis
Propionibacteria	Butyrate, propionate	SCFAs inhibit HDACs and thus, cell proliferation; Butyrate interacts with GPR109A on host cells and promotes the differentiation of Treg cells along with the activation of macrophages and T cells which release anti-inflammatory cytokines like IL10 and TGFβ
Most Gram negative bacteria	LPS	Anti-cancer immune response activation; TLR 4 signaling involved; inflammasome activation that results in IL-1β and TNF secretion
Lactobacilli spp.	Unknown	Anti-cancer immune response activation; activation of NK cells or DCs or TH1 response
Fusobacterium	Saliva	Might increase production of ROS & inflammatory cytokines; tumor immune microenvironment modulation; infiltration of myeloid cells in intestinal tumors.
Lactobacillus casei	Ferricrome	Apoptosis in cancer cells via JNK pathway
Salmonella enterica	Monophosphoryl lipid A (MPA)	Used as adjuvant in vaccine formulations against anti-cervical cancer
S. typhimurium	Flagellin	Production of interferon-γ (IFN-γ) by NK cells stimulation
Listeria spp.	Direct killing	Activates NAD phosphate oxidase; high ROS levels

Table 3: Bacteria with tumoricidal activities [5], [29]

4. Discussion

4.1 Cancer Therapy Regimes Using Microbiota

In 1984 it was observed that ulcers and cancers pertaining to stomach had a single bacterial type as the causative agent i.e. Helicobacter pylori; characterized as a class I carcinogen by the World Health Organization (WHO) [9]. The corollary of this was the role played by commensal microbiota in cancer development. The fact that most of the people carrying this particular bacteria do not exhibit symptoms and that it also protects from other cancer types, stresses the point that commensals aren't always causing pathological conditions [22]. Some microbes also help suppress tumor.

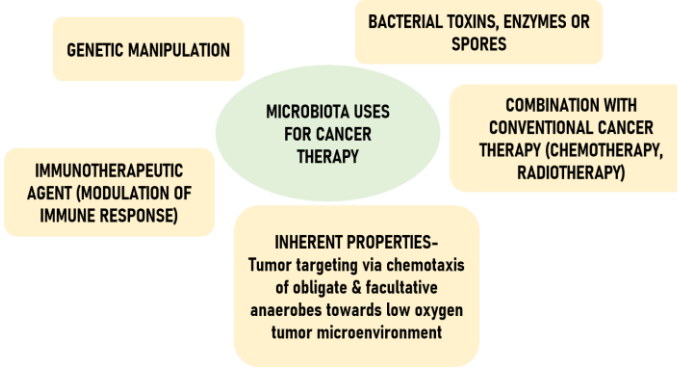

Figure 4: Modulation of the microbiota of the host to assist cancer therapy

4.1.1 Genetic manipulation of bacteria

Bacteria can be engineered genetically to achieve tumor targeting. In recent times, bacteria have been made to secrete proteins including toxins, cytokines, tumor antigens, and apoptosis inducing factors at tumor sites. A research group engineered E. coli and S. typhimurium strains to secrete Cytolysin A under a constitutive or an inducible promoter activated by arabinose. When induced, lysis releases the bacterial content along with the protein into the tumor microenvironment. Moreover, by deleting virulence genes (attenuation) from toxic strains, many safer strains have been developed. For example, VNP20009, an S. typhimurium strain was created by deleting msbB and purI genes that modified its LPS such that TNF induction was compromised. The strain was successful in mice but could not clear phase I trials in human cancer patients. Therefore, to retain the anti-tumor effect and be non-toxic, via deletion of pagP, pagL, and lpxR genes, yet modified LPS had a high affinity for TLR4.Nontoxic Salmonella mutant strains (relA- and spoT-) were made by manipulating endotoxin-associated genes. Unable to synthesize ppGpp, a peptide involved in toxin gene expression, the strain exhibited negligible toxicity and high anti-tumor effects (Duong et al., 2019). Furthermore, bacterial cancer therapy can be explored using various prokaryotic and eukaryotic expression systems for drug delivery. For instance, attenuated S. typhimurium (VNP20009) strain when administered along with E. coli expressing cytosine deaminase (pro-drug converting enzyme) showed conversion of non-toxic 5- florocytosine (5-FC) into chemotherapeutic 5-florouracil (5-FC) in the patients.Cytokines are known immune-modulators and have been expressed in bacteria for anti-cancer effects. Mouse with hepatocellular carcinoma (HCC) when orally administered with S. Typhimurium Ty21a strain expressing IL-2 had an inhibiting effect [29].All things considered, bacteria provide promising avenues for cancer treatment when genetically manipulated.

4.1.2 Tumoricidal agents generated by bacteria

As elucidated in Table 3, various agents secreted by commensals have the inherent potential to suppress tumor either directly or indirectly via immune system modulation.

4.1.3 Combination with conventional cancer therapy to enhance efficacy

Commensals calibrate systemic immunity with consequences in conventional tumor therapies mainly, chemotherapy and immunotherapy.

(a) Chemotheraphy

If gut microbes affect anti-cancer therapies then the therapeutic efficacy of chemotherapeutic agents might lie in microbiota modulation. Cyclophosphamide (CTx), is an alkylating cancer drug which promotes T-cell immunity via induction of Th17, Th1 cells, and induces cancer cell death. Given this drug, barrier disruption resulted in commensal infiltration into lymph nodes and spleen promoting the anti-cancer efficacy. Germ-free or antibiotics treated mice showed reduced anti-tumoral response. Hence, it was concluded that a lack of healthy gut microbiota leads to reduced anti-tumor immunity [14].Oxaliplatin is a platinum-based chemotherapeutic agent that forms DNA cross-links and drives anti-cancer T cell immunity. In response to platinum salts, microbiota primes for ROS production assisting the chemotherapeutic response [12].

(b) Immunotheraphy

Immunotherapy relies on substances made in the body or given from outside to boost the body's defense system for fighting cancer. CpGoligodeoxynucleotides (ODN) are abundant in bacterial DNA and are ligands for cells bearing TLR 9. The bacteria assist in TNF production by myeloid cells [12]. In combination with inhibitory IL-10 antibodies, CpG-ODN is given in immunotherapy [14].Programmed cell death protein-ligand 1 (PD-L1) antibody therapy is given in many cancer types. PD-L1 is expressed by antigen presenting cells (APCs) while PD-1 is expressed by activated T cells [12]. Analysis of the fecal microbiome of melanoma patients showed increased *Akkermansia* and *Alistipes*phyla and transplanting *Akkermansia* from patients to germ-free mice increased PD-1 blockade response to suppress. *Bifidobacterium*activates DCs to cause improved CD8+ T cell priming and accumulation in the tumor microenvironment; linked with improved tumor control in melanoma patients. Therefore, the anti-tumor response of PD-L1 antibody therapy can be modified by the microbiota [5].

Cytotoxic T lymphocyte antigen 4 CTLA-4, an immunomodulatory molecule expressed on CD4+ and CD8+ T cells blocks T cell activation and proliferation [30]. Anti-CTLA-4 monoclonal antibody i.e. ipilimumab, blocks CTLA-4 and has its anti-tumor effects enhanced by microbes especially *Bacteroidesthetaiotamicron*and *B. fragilis* [12]. A study showed suppression of sarcoma in CTLA blockade when the microbiome was enriched in *B. fragilis*[5]. Even in this case, the germ free or antibiotics treated mice did not respond to CTLA-4 blockade [12].Accordingly, to enhance antitumor immune responses, microbiota should be considered while giving immunotherapeutic agents that target T cell regulatory pathways.

4.1.4 Fecal Microbiota Transplantation

Fecal microbial markers show promise for cancer prognosis given aid from metagenomic studies. The people who initially did not benefit from the immunotherapy, after receiving fecal matter from a donor who responded to the treatment, did exhibit tumor reduction. In 2018, a woman with bladder cancer developed diarrhea like side effects after receiving checkpoint inhibitors but the symptoms resolved after one or two stool transplants from a healthy donor [12]. But chances of infection are there when transplanting poop, therefore rationally deciding the bacterial consortium would be a wiser way of treatment [31].

4.1.5 Probiotics

Most of the cancer therapies can cause microbial dysbiosis leading to mucositis or diarrhea like symptoms. To alleviate such symptoms, probiotics are given to the patients to repopulate the gut with healthy bacteria. Probiotics containing *lactobacilli* species are given to immunocompromised patients. Probiotic containing yogurt has proven useful in controlling stomach pathogens like *E. coli* and *H. pylori*. *Lactobacillus rhamnosus GG*is awell-established probiotic model in cancer. It is a gut resident bacterium with anti-inflammatory effects and helps establish gut bacterial homeostasis. With the benefits known well, probiotics also offer some shortcomings such as increased infection risk and antibiotic resistance. Various clinical trials are ongoing to test the efficacy for the use of probiotics in cancer patients [5].

4.1.6 Diet modulation

Using our diet at the forefront, the gut microbiome can be manipulated owing to the role played in cancer risk. A study when compared CRC risk in rural Africans and African Americans, the former population exhibited higher levels of *Prevotella*spp. and butyrate as compared to the latter one which hadhigher *Bacteroides*spp. and SBAs. The differences have been explained because of the difference in diet. RuralAfricans had higher resistant starch intake and African Americans had higher meatand fat intakes [8]. Dietary choices can affect cancer risk by promoting health and preventing disease.

5. Conclusion

Having understood the metabolic, physiologic, and molecular aspects of the host that get altered due to microbial interference, cancer intervention by dint of microbe modulation will be more promising if all the aspects of microbial-immune relation are clear. Personalized medicines are a vast area of research. What is more promising is the development of microbial biomarkers for detecting disease status in multiple cancer types. But large cohort studies are required before effective diagnostic or prognostic tests can be developed.

6. Future Scope

Future therapeutic studies should include microbiota as a central component contributing to diseased state and the patients should be stratified based on microbiome, genotype and also the geographical factor. In order to gain positive outcomes from probiotics administration and fecal transplants; clinical, genetic, and pathophysiological background of the patient should be known thoroughly.

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