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Unraveling Microbiome: The Role of Microbiota in Patients' Response to Oncological Treatment and Its Influence on Host-biochemistry

Elisha Apatewen Akanbong[1,a,*], Ali Senol[2,b], Mert Sudagıdan[3,c], Alparslan Kadir Devrim[2,d]

[1] Kırıkkale University, Institute of Health Sciences, Veterinary Biochemistry, Kırıkkale, Turkey
[2] Kırıkkale University, Faculty of Veterinary Medicine, Department of Biochemistry, Kırıkkale, Turkey
[3] Konya Food and Agriculture University, KIT-ARGEM R&D Center, Konya, Turkey

[a]ORCID: 0000-0002-2556-7236; [b]ORCID: 0000-0003-4080-7776; [c]ORCID: 0000-0002-3980-8344;
[d]ORCID: 0000-0002-3293-7290

*Corresponding Author
E-mail: akanbongelisha605@gmail.com

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Abstract

Despite the implication of viruses and bacteria in oncogenesis, commensal microbes play a beneficial role in anticancer therapy. Microbiota regulates responses made to various types of cancer chemotherapy by affecting the mechanism of action and toxicity of these therapies. From birth, microbiota confers innate and acquired immune responses and its role in modulating health and diseases has recently been appreciated. Increasing evidence also suggests that microbiota affects the pattern of body energy balance thus; they have a correlation with obesity, obesity-related complications and insulin resistance. Hence, microbiota has attracted the interest of researchers. However, there is little, if any, robust evidence-based review on their role in patients' response to cancer therapy and their influence on host-biochemistry thus, this review aimed to achieve that.

Keywords: Biochemical parameters, cancer chemotherapy, commensal microbes, microbiome, microbiota.

INTRODUCTION

Microbiome, although, frequently used interchangeably with microbiota, are not the same. Microbiota, since its coining in 2001, is used to describe the ecological community of microorganisms (symbiotic, commensal, pathogenic) whiles the microbiome encompasses genomes and all products of microbiota within the body (Orlandi et al., 2019). In other words, the microbiome is a more holistic, complex and diverse ecosystem pertaining to all host-associated microorganisms inhabiting epithelial barriers throughout the body and this includes bacterial microbiome (bacteria), archael microbiome (archaeal), virome (eukaryotic virusesand bacteriophages), mycobiome (fungi) as well as meiofauna (helminthic worms and unicellular protozoa) (Zitvogel et al., 2018). The microbiome is acquired via birth by means of vertical transmission (Zitvogel et al., 2018) and stays exposed to change by environmental factors such as nutrition throughout life. Also, microbiota influence brain and body development (Smith, 2015)as organisms raised in a controlled environment, that prevents the growth of microbiota (germ-free organisms) have poorly developed physiological conditions such as innate immunity (Raza et al., 2018). Mainly, it ensures homeostasis in the body especially, in the oral cavity, gastrointestinal tract, vagina and skin and this has a correlation with health status. A disruption in the microbial balance causes "dysbiosis" which subsequently leads to pathological conditions such as a chronic inflammatory disorder (Zitvogel et al., 2018). Following the tremendous discovery made by Williams B. Coley where anti-cancer responses were stimulated in cancer patients injected with heat-inactivated *Streptococcal* microorganisms, anti-cancer immunotherapy, in other words, the role of the immune system as a modulator of tumor proliferation/growth gained the attention of researchers (Bashiardes et al., 2017). According to Zitvogel et al. (2018), from birth, microbiota confers innate and acquired immune responses and its role in modulating health and diseases has recently been elucidated.

Cancer has been described as the second leading cause of death worldwide and it occurs as a result of intracellular accumulation of spontaneous mutations during DNA replication, combined with other environmental factors and lifestyle habits over a long period of time (Tomasetti and Vogelstein, 2015; Ashford et al., 2015). Environmental factors such as ultraviolet radiation, toxic substances and infectious agents among other factors have been termed to be cancer predisposing factors (Anand et al., 2008). Following tobacco, infections maybe the next important preventable cause of cancers and the interest in infectious causes of cancers has ebbed and flowed (Kuper et al., 2000a). The microbiome has been reported to be involved in the initiation and exacerbation of various forms of cancers at epithelial barriers (Zitvogel et al., 2018) and in sterile environments (Dzutsev et al., 2015). Emphasizing the involvement of suitable microenvironments in carcinogenesis is the demonstrated carcinogenic pathway of the first discovered oncovirus, *Rous sarcoma* virus which induced in adult birds cancer at sites of injection or injury and could not induce cancer in sterile embryos. In addition to this, one-quarter of the skin cells contained in aged and sun-exposed eyelids having clonally expressed carcinogenic mutants similar to those in squamous cell carcinoma but, maintain normal dermatological physiology without instigating

carcinogenic effects (Dzutsev et al., 2017). Moreso, epidemiological studies based on the analysis of oral, fecaland tissue samples to ascertain the role of microbiota and dysbiosis in carcinogenesis reported the only bacterium recognized by the International Agency for Research on Cancer (IARC) as a group I human carcinogen, *Helicobacter pylori (H. pylori)* as the underlying etiology of stomach cancer (Dzutsev et al., 2017). Contrary to this, the anti-cancer response has been stimulated in superficial bladder cancer patients with intravascular injections of *Mycobacterium bovis (M. bovis)* (Bashiardes et al., 2017), therefore indicating microbiome triggered immune-anticancer response. In fact, the microbiome can either be tumor-suppressive or oncogenic (Fullbright et al., 2013; Gagnaire, 2017).

Despite the complexity and multi-factorial nature of carcinogenesis (cancer onset), there seems to be a lot of evidence on microbial carcinogenesis. Enhanced capacity of amino acid metabolism, lipid metabolism and digestive system has been observed in a treatment group having the highest population of beneficial bacteria (Liu, 2018). It implies that microbiotas also have an essential impact on the biochemistry of a host.

In healthy individuals, the abundance of various microbiota varies greatly in accordance with the host's immune system, environmental conditions, infections and use of antibiotics, as well as genetics and diet (Wirth et al., 2020) which the latter (diet) has emerged as an essential influencer of microbiota composition and function (Zmora et al., 2019). Also, mouse models have shown deterioration of gut microbiota by perturbing forces such as antibiotics and industrialized diets (Sonnenburg and Sonnenburg, 2014; Korpela, 2016; Schulfer et al., 2018). In addition to this, genetically similar populations have shown different microbiotas due to lifestyle differences such as farming practices and water sources (Fragiadakis et al., 2019; Jha et al., 2018; Gomez et al., 2016; Morton et al., 2015).

However, the distal gut microbiota of an adult human is typically dominated by two bacterial phyla; the *Firmicutes* and *Bacteroidetes* (Mahowald et al., 2009) and the relative variation in the abundance of these two bacterial phyla seems to be related to obesity. A relative greater abundance of *Firmicutes* has been reported in obese-leptin deficient mice as compared to the microbiota of lean-control mice. Similarly, when germ-free mice were colonized with the microbiota (*Firmicutes*) from an obese donor, they manifested an increase in body fat despite having the same feed intake. Obese-patients having much abundance of *Firmicutes* and significantly fewer abundance of *Bacteroidetes* compared with lean-controls, when subjected to the same diet as the controls, subsequently had a relative increase in *Bacteriodetes* which positively correlated with weight loss (Indiani et al., 2018). However, there is little, if any, robust evidence-based review on their role in patients' response to cancer therapy and their influence on host-biochemistry thus, this review aimed to achieve that.

MICROBIAL CARCINOGENESIS

Prior to the end of the 19th century, the discoveries made in medical microbiology proved the involvement of bacteria and their etiology for major diseases and this pragmatically contributed to improved treatment. Not surprisingly, this perhaps led to the birth of the idea that bacterial infections might be the cause of carcinogenesis. However, these observations did not take into consideration the long-time lag between the initiation of carcinogenesis and onset of the overt disease. Just as the bacterial infection would not be thought of as the cause of cystic fibrosis in patients, the presence of bacteria at sites of carcinomas does not necessarily mean they are the cause (Lax and Thomas, 2002). Notwithstanding that, cell transformation could have been caused by bacterium/bacteria long before the manifestation of cancer and bacterium/bacteria could have been cleared from the body long before its full effects were seen. Lax and Thomas (2002), further stated that the involvement of bacteria in carcinogenesis remains partly controversial since there is no clear evidence on the molecular mechanism(s) by which bacteria cause carcinogenesis. Aside from that, the recognition of *H. pylori*as the cause of gastric cancer is based on epidemiological studies thus, its role in the emergence of stomach cancer is not straightforward (Falk et al., 2000). The diverse genetic nature of humans further complicates it as humans exhibit different susceptibilities to *H. pylori*infection and stomach cancer development (Magnusson et al., 2001). Furthermore, animal models have been used to provide more convincing evidence of other diseases. However, animal models used to demonstrate *H. pylori*infection resulted in a pathological display different from that seen in the human infection (Lax and Thomas, 2002, Dağ et al., 2016). Contrary, a disruption of the repertoire of gut microbiota, which is often known as "dysbiosis", is linked to several pathological conditions, including cancer (Carding et al., 2015) and they may elicit carcinogenesis by causing immune evasion or inflammatory disorders, DNA damage or direct tissue damage, in the case of oncovirus infection (Curty et al., 2019) (shown in Figure 1.). Models of bacteria-mediated carcinogenesis of several microbial species including, *Enterococcus faecalis (E. faecalis)*, *Streptococcus gallolyticus (S. gallolyticus)*, enteropathogenic *Escherichia coli (E. coli)*,*Salmonella enterica (S. enterica)*, *H. hepaticus*and *Fusobacterium nucleatum (F. nucleatum)* have also been characterized in mice (Kostic et al., 2013; Sears and Garrett, 2014).*F. nucleatum* has been etiologically linked to carcinogenesis of sporadic colorectal cancer (CRC) and *E. coli* strains to harbor a genomic virulence island have been reported to be capable of causing DNA damage and chromosomal instability in the host (Wirth et al., 2020). Besides, archae have also been found in fecal samples of colorectal cancer patients (Mira-Pascual et al., 2014). Viruses such as *Hepatitis B virus (HBV)*, *Epstein-Barr virus (EBV)*and *Human papilloma virus (HPV)* have been accepted as carcinogenic owing to their direct mechanistic single gene-induced cell transformation effects (Kuper et al., 2000a). The chronic form of HBV infection is estimated to be the definite cause of hepatocellular carcinoma based a geographical correlation, cohort and case-control studies, as well as clinical and laboratory investigations (Kuper et al., 2000b). It is responsible for more than half of all liver cancer cases globally (Kuper et al., 2000a). Approximately, 2 billion people have been infected with HBV and about 350 million people are chronic carriers of the virus with the highest prevalence being in Asia and Africa. It can be transmitted vertically (from mother to infant) or sexually (Kuper et al., 2000a).

In HBV infection, the HBV-DNA may be integrated into the host genome which potentially results in insertional mutagenesis involving structural changes such as deletion of host DNA; translocation, duplication of HBV-DNA and amplification of host DNA (Robinson,

1994). The x protein coded for by the HBV-genome is responsible for the inactivation of *P53* and other tumor suppressor genes. It has been reported that, *x* protein may pay its carcinogenic quota via the transcription of methyl transferases, thus causing regional hyper-methylation of DNA which causes the silencing of tumor suppressor genes or may cause general hypo-methylation resulting in chromosomal instability. Hence, it plays a vital role in hepatocarcinogenesis. Aside from the anti-apoptotic effect of *x* protein, it also instigates a pro-apoptotic effect. These contradicting effects are yet to be explained (Kew, 2011). Despite the strenuous and pragmatic efforts made to explain the molecular effects of HBV genome, direct viral effects of HBV are unlikely to be the main causes of liver carcinogenesis since not all hepatocellular carcinoma tumors contain HBV integrands (Kuper et al., 2000a).

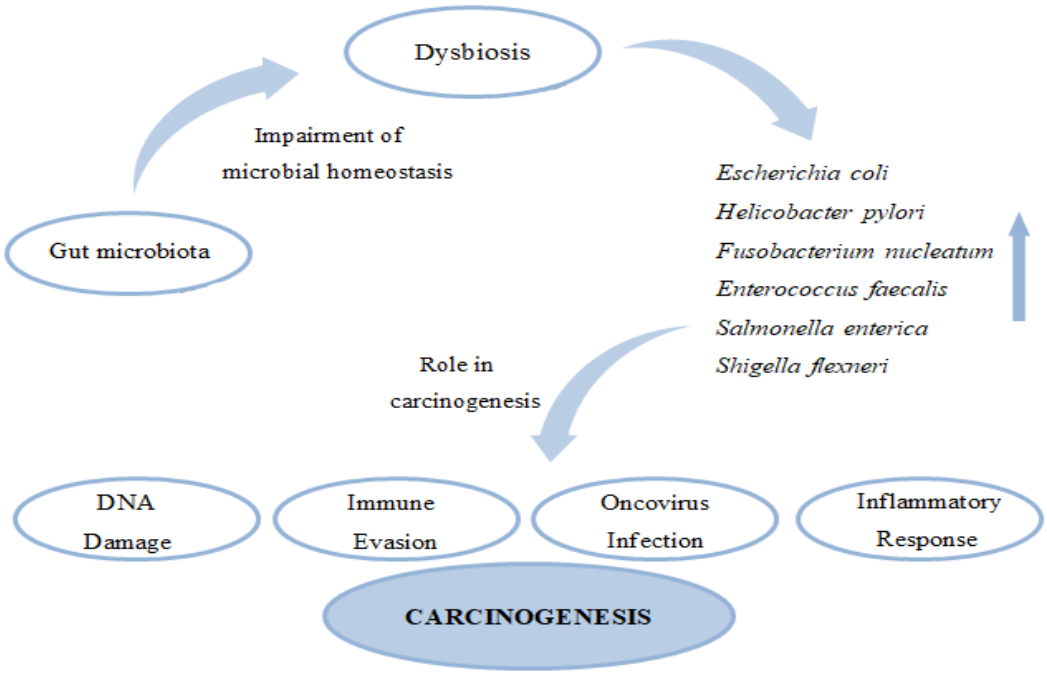

Figure 1. Illustrates dysbiosis-related carcinogenesis. Bacteria during gut dysbiosis can secrete toxins that may interfere with host cell growth, finally, predisposing the host organism to cancer development. Microbiota may elicit their carcinogenetic effect by either evading host-immune system or by triggering an inflammatory response, DNA damage or by directly instigating tissue damage (oncoviruses)

The member of the Herpes family of viruses, EBVwhich is made up of linear DNA, after infection, gains a circular shape to form EBV episomes with membranous envelope and glycoprotein spikes. Its genome codes for Epstein-Barr Nuclear Antigen 1 (EBNA1), EBNA2 and lymphocyte membrane-associated protein (LMP1) which may interfere with *P53* mediated apoptosis. The z protein which triggers the switch of EBV from latent to lytic infection can interact directly with tumor suppressor protein, *P53in vitro and in vivo.* EBV is an established carcinogen, having conclusive evidence with respect to non-Hodgkin's lymphoma and nasopharyngeal carcinoma (IARC, 1997). EBV has been associated with other forms of cancer including gastric carcinoma, but evidence surrounding these claims are weak (Kuper et al., 2000a).

Human papillomaviruses are sexually transmitted viruses and have high tissue-specificity and mainly infect the basal cells of the squamous epithelium in the genital tract, skin and upper respiratory tract. It has been associated with vulva, anus, penis, head and neck cancers but, it is emphatically known for its cervical cancer etiology (Vaccarella et al., 2006). Furthermore, a case-control study conducted by IARC has labeled HPV strains 16 and 18 as definite human carcinogens, and types 35, 45, 51, 52, 58 and 59 have been referred to be possibly related to carcinogenesis (Pisani et al., 1997). The carcinogenic effect of HPV is due to its ability to integrate into the host genome and code for the genes E6 and E7. These two genes are important for carcinogenesis and have been demonstrated to be capable of immortalizing the primary human genital keratinocytes *in vitro* (Pao et al., 1996). According to Pao et al. (1996), its ability to code for the E6 and E7 genes emanates from the disruption of the E2 gene during integration into the host genome. In other words, E2 gene has an anti-carcinogenic effect.

RELEVANCE OF MICROBIOTA IN CANCER THERAPY

Despite the implication of viruses and bacteria in oncogenesis, commensal microbes play a beneficial role in anticancer therapy (Perez-Chanona and Trinchieri, 2016). Anti-cancer treatments may evoke a tumor-destructive immune response by altering the microenvironment (Shirota et al., 2012) and in a study conducted, subcutaneous tumors failed to respond to immunotherapy and platinum chemotherapy after antibiotics treatment, which is a microbiota perturbing force (Iida et al., 2013). This suggests that microbiome plays a crucial role in the efficacy of a number of anticancer therapeutic approaches (Sivan et al., 2015; Iida et al., 2013; Viaud et al., 2013). Microbiota regulates the responses made to various types of cancer chemotherapy by affecting the mechanism of action and toxicity of these therapies. In view of this,

interventions on microbiome may be pivotal to improving toxicity that may emerge from anti-cancer therapy as well as improving the efficacy of anti-cancer therapies (Nayak and Turnbaugh, 2016; Fessler and Gajewski, 2017). This can be substantiated using the crosstalk between gut microbiota and myeloid cells for the production of reactive oxygen species (ROS) in the case of oxaliplatin and its induction of an anticancer T cell response in the case of cyclophosphamide (Roy and Trinchieri, 2017).

In the study conducted by Iida et al. (2013), the tumor-infiltrating myeloid-derived cells of antibiotics-treated or germ-free mice resulted in lower production of cytokine and tumor necrosis after CpG oligonucleotide treatment and deficient production of ROSand cytotoxicity after chemotherapy, thus, responded poorly to cancer therapy. In this light, an intact commensal community (microbiota) is vital to the success of cancer therapy. Viaud et al. (2013) also demonstrated the relevance of microbiota in response to cancer therapy using tumor-bearing germ-free mice (mice treated with antibiotics to kill Gram-positive bacteria). According to the study, the mice had reduced "pathogenic" T helper 17 (pT_H 17) and their tumors were resistant to cyclophosphamide. However, after an adoptive transfer of pT_H 17 cells, the antitumor efficacy of cyclophosphamide was restored. Consequently, Gram-positive bacteria shape the efficacy of cyclophosphamide since they stimulate the generation of pT_H 17 cells as well as memory T_H 1 immune response (Viaud et al., 2013). Oral administration of *Bifidobacterium* alone yielded a tumor control at a degree same as that of programmed cell death protein 1 ligand (PD-L1)-specific antibody therapy, and when administered concomitantly with PD-L1, they nearly abolished tumor outgrowth (Sivan et al., 2015). *Lactobacillus rhamnosus* GG (LGG) is a gut bacterium having anti-inflammatory effects (Khailova et al., 2017; Wang et al., 2017; Fong et al., 2016) and has been studied mostly as a probiotic due to its anti-inflammatory effect (Lee et al., 2014). LGG demonstrated a preservative effect on gut microbiota balance and on intestinal epithelial barrier functionality in radiation-mediated gut epithelial injury in animal models (Chang et al., 2018; Riehl et al., 2018; Zhang et al., 2017) and its administration in cancer patients has been pictured to have several potential benefits (Banna et al., 2017). Additionally, several *in-vitro* studies in tumor models including, colorectal, ovary and breast cancer tumor models have proven that, LGG has an anti-metastatic effect (Orlando et al., 2016; Nouri et al., 2016; Zhao et al., 2017). Also, treatment with LGG reduced tumor mass by modulating gut commensal microbiome in rat dimethyl hydrazine-induced colon cancer (Cai et al., 2016). Moreover, oral administration with *Lactobacillus casei* reduced the recurrence of superficial bladder cancer (Aso and Akazan, 1992). Also, according to Soyucok et al. (2020), the lactic acid bacterium *Lactobacillus helveticus* plays an essential role in the hydrolyses of milk proteins into bioactive peptides. Soyucok et al. (2020) further stated that, *L. helveticus* contributes essentially to health promotion as it modulates immune responses and intestinal microbiota by preventing gastrointestinal infections.

Furthermore, an evaluation of bacterial composition conducted on animals treated with CpG-ODN/a-IL-10 ab reported different bacterial species that correlated with the immune response against the tumor. For instance, the presence of the gram-negative *Alistipes genera* in the feces positively correlated with TNF production in the tumor (Farrokhi, 2019). In view of this, it can be concluded that *Alistipes genera* provided an anticancer response by contributing to an increase in the number of TNF-producing myeloid cells in the tumor tissue. Additionally, Microbiotas may provide a positive response to cancer therapy through the production of SCFAs. SCFAs such as butyrate and propionate have been demonstrated to have an anti-cancer effect in CRC and lymphoma by inhibiting host's tumor cells histone deacetylases (HDACs) (Wei et al., 2016; Jan et al., 2002), which are responsible for regulating the expression and activity of proteins involved in the initiation and progression of cancer (Glozak and Seto, 2007). The interaction between bacterial microbiota and anticancer chemotherapeutics can affect the efficiency of these anticancer chemotherapeutics. For instance, the activity of 10 out 30 chemotherapeutics has been reported to have been suppressed in the presence of nonpathogenic bacterial species *E. coli* and *Listeria welshimeri* (Lehouritis et al., 2015). This further suggests that microbiota modulates cancer therapy.

It is becoming evident that, aside from the impact of gut microbiotas on cancer therapy, cancer therapy may in turn affect gut microbiotas. Chemotherapy may affect metabolic pathways by causing profound dysbiosis (Alexander et al., 2017; Montassier et al., 2015). In the course of chemotherapy, antibiotics are frequently administered to patients and this should be given much attention as it has been proven that; concurrent administration of antibiotics negatively affects the outcomes of cancer immunotherapy (Derosa et al., 2018). The administration of microbial consortia (probiotics) in an attempt to improve human health is nothing new, as efforts are still in progress to modulate the gut microbiota since the early 1900s when Metchnikof theorized that, administration of microbes could have beneficial effects (Podolsky, 2012). However, some preclinical and clinical studies conducted on commercial probiotics reported varied results. Although some reported positive potentials of the probiotics (Zhu et al., 2011; Appleyard et al., 2011), other studies with the same probiotics reported deleterious effects of the probiotics, with increased tumor penetrance and multiplicity (Arthur et al., 2013). Theoretically, the timing of probiotic administration, among other factors was explained to have contributed to the differences in effect. Clinical trials to study the impact of probiotics administration have been done in cancer patients but most of these studies focused on their impact on changes in microbiota composition and not specifically ascertain their influence on outcomes of cancer therapies (Helmink et al., 2019). Such report includes the administration of probiotics to patients with CRC which resulted in an alteration in the gut microbiota by increasing the abundance of butyrate-producing microbes in mucosal and fecal samples following administration (Hibberd et al., 2017). This insinuates that probiotics still have a promising effect of restoring microbiota composition or correcting dysbiosis. However, studies involving the impact of commercially available probiotics on microbiota should not be neglected as the content of commercially available probiotics may vary significantly from what is advertised (Kolaček et al., 2017; Morovic et al., 2016).

EFFECTS OF MICROBIOTA ON BIOCHEMICAL PARAMETERS

Increasing evidence suggests that gut microbiota may influence weight-gain via several inter-dependent pathways such as short-chain fatty acids signaling, modification of behavior, controlling of appetite,

modulating of inflammatory responses within-host (Bliss and Whiteside, 2018) and energy harvesting (Ley et al., 2006). Also, dysbiosis may also lead to altered host inflammation status as well as liver disease (Boulangé et al., 2016; Minemura and Shimizu, 2015) as indicated in the summary(shown in Figure 2). In terms of energy, a balance should exist between energy intake and expenditure. An imbalance where the former (intake) exceeds the latter (expenditure) will result in obesity, which is a complex and global epidemic disease (Bliss and Whiteside, 2018), obesity-associated complications and insulin resistance (Shen et al., 2013; Prieto et al., 2018). Proteobacteria, are Gram-negative bacteria with lipopolysaccharides (LPS) in their outer membranes. They have been reported by Prieto et al. (2018) to have a correlation with ghrelin levels. Chang et al. (2003) reported that LPS directly stimulated the gastric mucosa to synthesize and secret ghrelin in rats, which is considered as a therapeutic effect against the endotoxic shock produced by LPS. In this context, proteobacteria or LPS-containing bacteria can be termed to have a positive correlation with ghrelin.

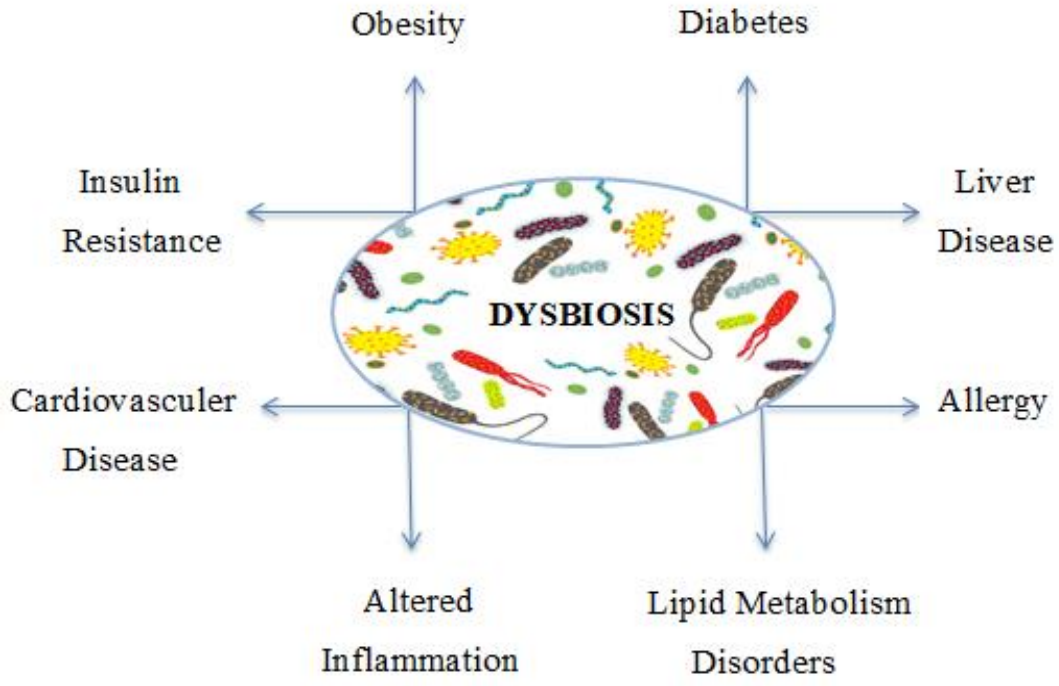

Figure 2. Dysbiosis may cause many diseases by changing intestinal permeability and the biochemical profile of the host.

Prieto et al. (2018) further stated that *Desulfovibrio* and *Alistipes indictintus*, which are LPS-containing bacteria also had a correlation with plasmatic insulin levels and hence they are involved in glucose intolerance. LPS-induced metabolic endotoxemia has been reported to be the first step in the development of insulin resistance diabetes (Type II diabetes) and this has been demonstrated with an experimental infusion of LPS which resulted in hyperglycemia and hyperinsulinemia (Cani et al., 2007). Leptin is a hormone secreted by fat cells to inhibit feeding by eliciting its effect via the hypothalamic pathway in order to achieve energy balance and body weight regulation (Jéquier, 2002). Gut microbiota has been reported to have crosstalk with this hormone since it is capable reducing hypothalamic sensitivity to leptin by increasing leptin resistance-associated suppressor cytokine signaling 3 (Schéle, 2013). Medina-Vera et al. (2019) reported that, type 2 diabetic patients exhibiting intestinal dysbiosis characterized by an increase in *Prevotella copri*, with a dietary intervention independently of antidiabetic drugs had decreased *Prevotella copri* with an increased *Faecalibacterium prausnitzii* and *Akkermansia muciniphila* (two bacteria known for their anti-inflammatory effects). Additionally, patients also exhibited a significant decrease in glucose, total and low-density lipoprotein (LDL) cholesterol, free fatty acids (FFAs), triglycerides, glycosylated hemoglobin (HbA1c) and an increase in antioxidant activity. This further elaborates the biochemical parameters modulating the effect of the microbiota.

In addition, certain commensal bacteria are known to produce essential micronutrients such as vitamin K and other components of vitamin B. Some members of the family *Bacteroides* have been reported to be capable of synthesizing the anti-diabetic linoleic acid, catabolizing secondary bile acids and capable of breaking down phenolic compounds. Furthermore, some gut microbiotas are capable of modifying some amino acids by means of decarboxylation into signaling molecules such as; histidine into histamine and glutamate into gamma-aminobutyric acid (GABA) (Mohajeri et al., 2018). Additionally, the commensals of the gut can also produce hormone-like metabolites known as short-chain fatty acids (SCFAs) by fermenting dietary fibers in the large intestine. These SCFAs when produced, they are transported via the bloodstream to the liver, where they are used as a source of energy. SCFAs also play an essential role in controlling the metabolism of glucose and lipid by affecting the

secretion of a peptide hormone, peptide YY (PYY) and glucagon-like peptide 1 (GLP-1) (Clarke et al., 2014).

CONCLUSION AND RECOMMENDATION

Microbiota is an essential integral human component that keeps evolving with time. Its role in response to cancer therapies or efficacy of cancer therapies is inevitable. Also, an intact microbiota is important for the maintenance of a healthy host-biochemistry. Although viral microbiotas such as HBV, EBV and HPV have been reported as carcinogenic, there are still discrepancies on the carcinogenicity of bacteria microbiome. Subsequent studies should be conducted on the influence of microbiota on response to cancer therapy and restoration of gut microbiota after treatment. Studies should be conducted on the molecular mechanism(s) of bacteria carcinogenicity. Further exploration should be done on the anti-carcinogenic effects of the *x* protein coded for by the HBV genome and the E2 gene which is also coded for by the HPV genome. Further studies should also be conducted on the correlation between microbiota and biochemical parameters.

Conflict of Interest

The authors declared that there is no conflict of interest.

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