Correlation between Dysbiosis of Gut Microbiota and Human Colorectal Cancer

Chae Rin Oh
All Saints University School of Medicine, Dominica

Abstract—The exquisite balance between beneficial bacteria and harmful bacteria is maintained in the gastrointestinal tract. If meat-eating habits persist, the population of harmful bacteria that cause carcinogens increases in the human gut. Some carcinogenic substances are produced in tryptophan metabolism, which degrades proteins. Others are produced during the process of producing fat-decomposing bile. The beneficial bacteria stimulate the activation of immune cells and control the population of harmful bacteria. However, when the population of beneficial bacteria with the ability to regulate immune cells is reduced. At this time, the population of harmful bacteria increases sharply, it is generated carcinogenic agents causing colon cancer.

Keywords—Gut Microbiota, Short-Chain Fatty Acids (SCFA), β-Glucuronidase (β-G), Beneficial Bacteria, Harmful Bacteria, Gastrointestinal Tract, Symbiosis, Dysbiosis.

1. INTRODUCTION

The human body is inhabited by a vast number of bacteria, archaea, viruses, and unicellular eukaryotes. It is estimated that the human microbiota contains as many as 10^{14} bacterial cells, a number that is ten times greater than the number of human cells present in our bodies. Gut microbiota are comprised of 500-1000 species of microbe. The phyla Firmicutes and Bacteroidetes predominantly constitute the healthy gut microbiota. Gut microbiota provides its host with a physical barrier to incoming pathogens by competitive exclusion, such as occupation of attachment sites, consumption of nutrient sources, and production of antimicrobial substances. It also stimulates the host to produce various antimicrobial compounds. From an immunological perspective, microorganisms are viewed as pathogens by the host immune system that recognizes and eliminates them. However, majority of the gut bacteria are non-pathogenic. The gut commensals predominantly aid in nutrient metabolism and prevention of colonization of pathogenic microorganisms. At the same time, the immune system has co-evolved to live in a collaborative relationship with the healthy microbiota, while serving its function to fight off invasive pathogenic microorganisms. Only 2 of them dominate the human gut microbiota: the Bacteroidetes and the Firmicutes, whereas Proteobacteria, Verrucomicrobia, Actinobacteria, Fusobacteria, and Cyanobacteria are present in minor proportions. This study aims to investigate a correlation between dysbiosis of gut microbiota and colon cancer development.

The Role of the Gut Microbiome

The gut microbiota protect the intestinal epithelial cells by inhibiting the intestinal immune system while sterilizing the immune system by activating the immune system instantly when bad pathogens enter into the intestine. The intestinal homeobox gene Caudal regulates the commensal-gut mutualism by repressing nuclear factor kappa B-dependent antimicrobial peptide genes. The gut microbiota is essential to the proper function and development of the host. Many bacterial species are implicated in metabolism of dietary fiber to SCFA (short-chain fatty acids), accounting for a significant part of the human energy source. The gut microbiota plays a pivotal role in the how fiber benefits health—and it’s mostly thanks to their host of molecular messengers, called SCFA. SCFAs are molecules produced by bacteria when they ferment dietary components (primarily fiber: non-digestible carbohydrates) inside the colon. Some of these molecules stay close to home in the gut, but others travel far and wide throughout the body. The formation of SCFA is the result of a complex interplay between diet and the gut microbiota within the gut lumen environment. Production of some of SCFA (short-chain fatty acids) such as butyrate, is not only important as an energy source for the host but also prevents the accumulation of potentially toxic metabolic by-products, such as D-lactate. Short-chain fatty acids (SCFA) are the primary end-products of fermentation of non-digestible carbohydrates (NDC) that become available to the gut microbiota. Members of the genus Bacteroides, which are the predominant organisms that participate in carbohydrate metabolism, perform this by expressing enzymes such as glycosyl transferases, glycoside hydrolases, and polysaccharide lyases. The best example among these organisms is Bacteroides that is endowed with a genome that codes for over 260 hydrolases, which is far more than the number encoded by the human genome. It is reported that the pathogenesis and development of this disease were associated with intestinal flora. Human intestinal floras are important factors of intestinal environment. Host’s heredity and external environment in which the host exists would affect the balance of human intestinal microecology. The host’s health condition is also closely related to the balance of human intestinal microbiology. β-glucuronidase (β-G) is an acid hydrolase, its positive rate in E. coli is up to 97%, and it has a high specificity. β-G-mediated glucuronidation is the main pathway of detoxification in human body, while the activity of β-G carried by intestinal flora in colorectal cancer patients is obviously lower than that in healthy population.

Contributions of the Gut Microbiota to Colorectal Carcinogenesis

Intestinal bacteria can be categorized as Intestinal bacteria can be categorized as beneficial or harmful bacteria if they are
classified based on the influence on human health. The former is a bacterium that coexists with the colon epithelial tissue, and the latter is a bacterium that damages the colon epithelial tissue. The beneficial bacteria decompose sugars in foods to produce lactic acid and alcohol. The harmful bacteria decompose proteins and amino acids and produce bad substances in human body such as hydrogen sulfide and ammonia. The proportion of bacteria in gut is different for each person, but most of the beneficial bacteria are present, and there are some harmful bacteria. However, the increase in harmful bacteria has negative effects on health.

Most of bacteria in human body have a beneficial role to health while they also cause some opposite effects. They mainly play the beneficial role in human body under normal circumstances; however, they would cause a pathogenic effect when the human body environment changes, because balanced steady-state system may be destroyed to lead to the microdysbiosis.

Gut microbiota can be classified into Species (species) - Genus - Family - Order - Class - Phylum - Kingdom - Domain. Human gut microbiota are divided into four phylum. Gram-negative bacteria, Bacteroidetes and Proteobacteria, Gram-positive bacteria, Firmicutes and Actinobacteria. Bacteroidetes species and Firmicutes with Clostridia are predominant.

Meat-based diet may disturb the balance of intestinal microbiota and lead to excessive growth of pathogens while decreasing the proportion of beneficial bacteria such as Lactobacilli and Bifidobacteria. Members of Lactobacillus and Bifidobacteria genus inhibit growth and development of several pathogenic bacteria by producing lactic and acetic acids as their major metabolic end products and less amounts of succinic, formic acids and ethanol. The main gut bacterial phyla, are Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Verrucomicrobia, and Fusobacteria. Firmicutes are gram-positive bacteria, including the large class of Clostridia and the lactic acid bacteria, while Actinobacteria are gram-positive bacteria, including Colisella and Bifidobacterium spp. Lactic acid bacteria and Bifidobacteria are two important types of gut bacteria. Lactobacillus and Leuconostoc spp. are the main lactic acid bacteria found in the human intestine. Gut bacteria play an important role in human health, including contributing to the host gut defense system and helping the gut to maintain normal function, while the host can influence its composition. In the process of decomposing large amounts of fats from meat, cholesterol and bile acid are secreted from the gastrointestinal tract.

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**TABLE 1. Gut bacteria**

<table>
<thead>
<tr>
<th>Beneficial bacteria</th>
<th>Harmful bacteria</th>
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<tbody>
<tr>
<td>Lactobacillus</td>
<td>Escherichia</td>
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<tr>
<td>Bacteroides</td>
<td>Clostridium</td>
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<tr>
<td>Eubacterium</td>
<td>Salmonella</td>
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<tr>
<td>Citrobacter</td>
<td>Streptococcus</td>
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<tr>
<td>Ruminococcus</td>
<td>Prevotella</td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>Bacteroides fragilis</td>
</tr>
<tr>
<td>Clostridiales</td>
<td>Staphylococcus</td>
</tr>
</tbody>
</table>

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GB: Gut Bacteria. PBA: Primary Bile Acids. SBA: Secondary Bile Acids. DCA: deoxycholic acid. LCA: lithocholic acid

Excessive concentration of bile acids especially lipophilic secondary bile acids are cytotoxic causing reactive oxygen species-mediated damage to the cells. Resistance to this apoptosis and accumulation of mutations leads to progression of cancer. The gut microbiota produces metabolites from a large range of molecules that host's enzymes are not able to convert. The primary bile acids, chenodeoxycholic acid (CDCA) and cholic acid (CA), are converted to the secondary bile acids, lithocholic acid (LCA) and deoxycholic acid (DCA) by the gut microbiota. Therefore, bile acids metabolism by the gut microbiota may promote disease development depending on the quantity and type of secondary bile acids produced. The dehydroxylation of chenodeoxycholic acid lead to lithocholic acid which is toxic to the gastrointestinal cells and is linked to colon carcinogenesis.

Similarly, high levels of deoxycholic acid in blood and feces are associated with increased risks of colon cancer. Increased carbohydrate metabolism further increases fat production in the liver. Fat accumulation of intestinal tissues due to obesity results in the proliferation of intestinal bacterial Firmicutes. Bile acids are normal components of the luminal contents of the gastrointestinal tract, where they enable absorption of lipids, cholesterol, and fats. In essence, they act as a physiologic detergent and regulator of intestinal epithelial homeostasis in the gastrointestinal tract. LCA, a secondary bile acids, also constitute a rare example of toxic antibiotics. In fact, bile acids were first proposed as a potential tumor-promoting agent. At high physiologic concentrations, bile acids can cause oxidative stress, DNA damage, and mutation. Furthermore, frequently repeated and prolonged exposure of tissues to high physiological levels of bile acids can lead to the generation of genomic instability, ultimately, cancer.

Dietary obesity induces alterations of gut microbiota, thereby increasing the levels of deoxycholic acid (DCA), a gut bacterial metabolite known to cause DNA damage. DCA produces reactive oxygen species that can cause DNA damage. Microbial byproduct DCA was generated as...
secondary bile acids through Clostridium in vivo. DCA is known to induce reactive oxygen species production and causes DNA damage as a potential carcinogen. DCA content is increased in feces from humans who consumed a high-fat diet. Gut microbiota plays a significant role in the development of carcinoma. Accumulation of cholesterol and bile acids in the liver and feces increased after feeding the mice with fat diet. But, accumulation of secondary bile acids such as DCA was dramatically reduced after the treatment with antibiotics, suggesting the critical role of the gut microbiota in the conversion of primary bile acids to secondary bile acids.

Collectively, bile acid metabolism by the gut microbiota promotes carcinoma. DCA can damage chromosomes and does so through its generation of reactive oxygen species (ROS). Severe DNA damage often triggers apoptosis (programmed cell death), which is an important mechanism to prevent the replication of cells with mutations that might result in cancer development. Indeed, to become malignant, cells must develop the ability to resist apoptosis. Bile acid-induced DNA damage might be able to resist apoptosis, and enable dangerous mutations to persist and facilitate neoplastic progression. DCA causes DNA damage and induces phosphorylation of proteins in the NF-κB signaling pathway in intestinal epithelial cells in vitro and in vivo.

A study shows that the generation of ROS mediates DCA-induced DNA damage and NF-κB pathway activation, and that DCA-mediated activation of the NF-κB pathway allows intestine epithelial cells to resist apoptosis in the setting of DNA injury, events that might contribute to neoplastic progression. It is used phosphorylation of H2AX as a marker for DNA damage. H2AX becomes phosphorylated on serine 139, then called gamma-H2AX, as a reaction on DNA double-strand breaks (DSB). H2AX becomes phosphorylated in response to various types of DNA damage, including single- and double-stranded breaks (DSBs). DSBs are among the most serious forms of DNA damage, because cells with persistent DSBs have been found to develop chromosomal abnormalities that can contribute to genomic instability and cancer formation. Persistent DSBs may cause chromosomal abnormalities including translocations and deletions and induce genomic instability, thus contributing to the tumorigenesis. Histone H2AX phosphorylation is a marker of double-stranded DNA break. DCA significantly increase H2AX phosphorylation in intestine epithelial cells, indicating that DCA may cause double-stranded DNA break. The lipid peroxidation radical, which is produced in the metabolism of the fat in the gastrointestinal tract may aggravate the carcinogenesis process. The end products of lipid peroxidation, 4-hydroxynonenal (4-HNE), have been considered to be a second messenger of oxidative stress. 4-HNE is a major bioactive marker of lipid peroxidation, due to its numerous biological activities resembling activities of ROS (reactive oxygen species). ROS are generated as by-products of cellular metabolism, primarily in the mitochondria and include free radicals such as superoxide anion, perhydroxyl radical, hydroxyl radical (·OH), nitric oxide (NO), and other species such as hydrogen peroxide (H₂O₂), singlet oxygen (¹O₂), hypochlorous acid (HOCl), and peroxynitrite (ONOO⁻). ROS can easily react with membrane lipids, causing an alteration of membrane permeability; with DNA, causing damage and genomic instability. Lipid peroxidation is the oxidative degradation of lipids. It is the process in which free radicals steal electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. The fatty acid radical is not a very stable molecule, so it reacts readily with molecular oxygen, thereby creating a peroxyl-fatty acid radical. This radical is also an unstable species that reacts with another free fatty acid, producing a different fatty acid radical and a lipid peroxide. Formation of ROS has been linked to cancer initiation and progression by inducing lipid peroxidation, DNA damage/mutation, and cell proliferation. Indeed, one of the most extensively LP products are the 4-hydroxynonenals (4-HNE) which modulate some signaling processes such as Akt pathway involved in cancer initiation and progression. If a lot of animal protein is introduced into the gastrointestinal tract by carnivorous habit, this allows to increases in the risk of cancer. Amino acids, a component of the protein, enter the tryptophan metabolism is processed by gut microbiota. The gut microbiota influences the health of the host, especially about gut immune homeostasis and the intestinal immune response. In addition to serving as a nutrient enhancer, L-tryptophan (Trp) plays crucial roles in the balance between intestinal immune tolerance and gut microbiota maintenance. Recent discoveries show that changes in the microbiota modulate the host immune system by modulating Trp metabolism. Tryptophan-derived indoles are involved in the host–microbe interaction in the intestine. The kynurenine (KYN) pathway is implicated in diseases such as cancer. Tryptophan is utilized in various metabolic routes including protein synthesis, and the kynurenine pathway. Perturbations in these pathways have been associated with cancer. L-Tryptophan (L-Trp) is a large neutral amino acid present in living organisms, precisely one of the 20 L-amino acids incorporated in proteins during the process of mRNA translation. Trp is primarily metabolized through two metabolic pathways: the kynurenine pathway (KP) and the serotonin pathway. On entering the kynurenine pathway, tryptophan is converted to N-formyl-L-kynurenine by tryptophan 2,3-dioxygenase and indoleamine 2,3-dioxygenase. Approximately 95% of the Trp ingested is degraded to kynurenine, kynurenic acid (KA), quinolinic acid, picolinic acid, and nicotinamide adenine dinucleotide (NAD) through KP, which is regulated by two rate-limited enzymes: tryptophan 2,3-dioxygenase (TDO) in the liver and indoleamine 2,3-dioxygenase (IDO) in extrahepatic tissues. IDO is the first and rate-limiting step in tryptophan catabolism along the kynurenine pathway. IDO is the enzyme able to metabolize tryptophan to kynurenine. IDO may play a role in colorectal cancer (CRC) pathophysiology. A study shows that IDO expression was particularly high in the neoplastic epithelium at the tumor tissue. Another study found a correlation between high density of IDO cells in tumor-draining lymph nodes and reduced 5-year survival rates in colon cancer patients.
Specifically, Trp is degraded to kynurenine, which is then largely metabolized to 3-hydroxykynurenine by kynurenine hydroxylase and marginally metabolized to anthranilic acid (AA) by kynureninase and KA by kynurenine aminotransferase. Furthermore, 3-hydroxykynurenine is mainly degraded to 3-hydroxyanthranilic acid by kynureninase and marginally degraded to xanthurenic acid by kynurenine aminotransferase. Through multi-stage enzymatic reactions, 3-hydroxyanthranilic acid is converted to quinolinic acid, pyridine carboxylic acids (such as picolinic acid, acetyl CoA), nicotinic acid, NAD\(^+\), and other active molecules. 3-Hydroxykynurenine (3-HK) is an endogenous oxidative stress generator. 3-hydroxyanthranilic acid (3-HANA) has also ambiguous characteristics which cause toxicity can produce DNA damage due to its interaction with metals and with the ability to generate hydroxyl radicals through Fenton’s reaction. Gut bacteria is a strong inducer of indoleamine 2,3-dioxygenase (IDO). Kynureninase is then metabolized to new compounds that can have toxic properties. The toxic effect of the intermediates 3-hydroxykynurenine and 3-hydroxyanthranilic acid involves the generation of \(\text{O}_2^{=}\) and \(\text{H}_2\text{O}_2\), which contribute to the oxidative processes. The carcinogens produced by micro-flora are as follows: Tryptophanase, decarboxylase, desulfurase, deaminase, uroase, azoreductase, nitrate reductase, nitroreductase, nitrosation, \(\text{7a}\)-hydroxylase, cholanonylhydrolase, steroid nucleus dehydrogenase, tyrosinase, lecithinase, \(\beta\)-glucosidase, \(\beta\)-glucuronidase, these produce carcinogenic substances are produced in the gastrointestinal tract. The normal gut microflora consists of bacterial species with morphological, physiological and genetic features that let it to colonize and multiply under particular conditions at certain sites, coexist with other colonizing microorganisms and competitively inhibit the growth of pathogenic bacteria. The colonic microflora is very rich and dominated by strict anaerobic bacteria such as Bacteroides, Fusobacterium, Clostridium and many others. However, it is conceivable that they include: alteration of the intestinal microflora; inactivation of carcinogenic compounds; competition with putrefactive and pathogenic microbiota; improvement of the host’s immune response; anti-proliferative effects via regulation of apoptosis and cell differentiation; fermentation of undigested food; inhibition of tyrosine kinase signaling pathways. The deconjugation of glucuronides in the intestine by bacterial \(\beta\)-glucuronidase (\(\beta\)-G) leads to the release of aglycones that are potentially carcinogenic substances. There are other fecal bacterial enzymes, including azoreductase and nitroreductase, which catalyze the liberation of procarcinogenic substances in the intestine. It is indicated that \(\beta\)-G from stool can catalyze procarcinogen to convert to carcinogenic substance, and \(\beta\)-G-mediated glucuronidation in human intestinal flora is the main pathway in detoxification of human body. The most notorious \(\beta\)-G is induced from Escherichia coli, Lactobacillus gasseri, and Ruminococcus gnavus.

The Dysbiosis of Gut Microbiota

The gastrointestinal tract is responsible for digestion. The small intestine absorbs carbohydrates and lipids. The colon sends the digested food out of the body. The gastrointestinal tract have a large amount of gut bacteria. It is divided into beneficial bacteria with intestinal epithelial cells and harmful bacteria attacking intestinal epithelial cells. The immune system in the gastrointestinal tract properly protect healthy body against harmful bacteria. The beneficial bacteria directly intervene in the differentiation and proliferation of cytotoxic T cells and regulatory T cells. In particular, in the gastrointestinal tract, the differentiation of immune cells is controlled by the increase or decrease of Clostridium species bacteria. If the number of The beneficial bacteria is reduced rapidly, the number of pathogenic bacteria increases sharply. The beneficial bacteria control the differentiation of immune cells, and the immune cells remove pathogenic bacteria. This is optimal conditions. Gut bacteria supply nutrients and energy by fermenting ingredients that are not degraded by digestive enzymes in the body. For example, antibiotic overuse, dietary habits, and stress cause gut bacterial imbalance, it is closely related to cancer. Recently, studies on symbiosis and dysbiosis between gut bacteria and host are under conduction.

The number of colon cancer patients in Korea is increasing rapidly. A typical western diet increases risk of of colon cancer. Many studies show that dysbiosis in gut microbiota is the cause of colon cancer. A comparison of gut microbiota ratio in colon cancer patients versus gut microbiota ratio in control group indicates the correlation between gut microbiota and colon cancer incidence. Gut microbiota ratio of colorectal cancer patients is much different from that of control group. Fusobacteria and Prevotella are found in high proportion in colorectal cancer tissues. Animal high-fat diets are resolved by digestive enzymes but promote the secretion of bile acids. Cholonic acid and cholesterol are converted into secondary bile acids and cholesterol metabolites by the enzymatic degradation of gut bacteria. This is a powerful cancer-causing factor that causes colon cancer. In animal high
protein esophageal metabolism, it is decomposed into amino acids and then transformed into tryptophan metabolites (indole, skatol, etc.), ammonia, phenol, amine and Nitroso compounds by corrupt microorganisms and acts as a carcinogen. Some study suggests a new hypothesis to explain the role of high-fat diet and gut microbiota at the molecular level in the development of colorectal cancer. Recently, Protein kinase C (PKC) is a adapter protein that is specific for differentiation and proliferation of cells. Carcinogens directly enhance the activity of PKC. Activation of PKC and cancer are highly correlated. Animal fat is degraded into diacylglycerol (DAG) by the action of gut microorganisms and bile acid, and the DAG is absorbed into the colon epithelium to increase the activity of PKC. Lactobacillus acidophilus symbionts inhibit the activity of fecal carcinogenic enzymes, β-glucuronidase, and nitroreductase. When Lactobacillus acidophilus milk was fed to patients with colorectal cancer, the concentration of bile acid in the feces decreased markedly. If we eat a lot of fermented milk products, lactic acid bacteria in the gastrointestinal tract, bacterial carcinogenic enzymes that cause cancer are constantly being produced. Among these enzymes, carcinogenic enzymes known so far are azoreductase, β-glucuronidase, and nitroreductase, which are mainly produced by gut parasites. These three enzymes play an important role in colon cancer development. A bacterial species found in the human gut may have important implications for treating colorectal cancer.

Lactobacilli and Bifidobacterium inhibit the development of colon cancer induced by harmful bacteria. Colon cancer is promoted by harmful organisms such as E. coli but can be inhibited by lactic acid bacteria such as Lactobacillus and Bifidobacterium. Lactic acid bacteria plays a very important role in the prevention of colon cancer among gut microflora. Dysbiosis of gut microflora causes colorectal cancer. Increased harmful bacteria such as pathogenic E. coli, toxic Bacteroides fragilis, and Streptococcus gallolyticus may be the cause of colon cancer. Clostridium cocoides and C. leptum group were more common in the feces of colon cancer patients, and Coriobacteria were increased, and Enterobacteria were decreased in colorectal cancer tissues compared to the surrounding non-cancer parts. Harmful bacteria can cause DNA damage, gene mutation, and chromosomal instability by promoting production of Nitric Oxide or reactive oxygen species to the host. Toxic substances produced by pathogenic gut bacteria are genotoxic. Some of the amines, metabolites of gut bacteria, are also carcinogenic. These amines, a risk factor for colorectal cancer, are produced through high-protein diets. Tumors harbor distinct microbial communities compared to nearby healthy tissue. In the tumor microenvironment, changes in microbial diversity occur. It means with changes in the abundances of commensal and pathogenic bacteria, including Fusobacterium and Providencia. Fusobacterium and Providencia, a novel tumor-associated agent in colorectal cancer.

II. CONCLUSION

Tumors as being composed of cancer cells, but tumors consist of cancer cells, non-cancer cells, and associated microbes, and therapeutic regimens may need to target all members of these cellular communities to be most effective. In a previous research, it is identified the bacterial species, called Fusobacterium nucleatum, as one of the most prevalent in colorectal tumors. It is found Fusobacterium DNA in both the primary and metastatic tumors. The tumors are [likely] benefiting from Fusobacterium—the Fusobacterium may be providing essential nutrients or growth signals to the tumor. The tumor appears to be providing the Fusobacterium with a suitable, immune-protected niche that helps it colonize and grow within the gastrointestinal tract.

In this study, we learned the need for new drugs that balance the harmful and lactic acid bacteria in the colon tissue to prevent colon cancer.

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REFERENCE

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