Gut Microbiome Drives the Development of Colorectal Cancers and Potential Therapeutic Interventions

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ABSTRACT
Multidrug resistance is a major problem that modern society must deal with. Bacteria that are dangerous to humans, fish, and farm animals are becoming increasingly resistant because of the widespread use of these drugs. In addition, factors including rising rates of infection and the prevalence of chronic conditions that respond well to antimicrobial therapy have contributed to a rise in antibiotic use. Since antimicrobial resistance is growing, measures must be taken to prevent the spread of illness and protect the health of humans and animals. Thus, there are several initiatives aimed at combating multidrug-resistant bacteria, including the use of cutting-edge methods like nano therapy, Crispr/Cas 9, and phage therapy. Furthermore, the progression of the disease and, therefore, an individual's health, are both directly correlated with the composition of the gut microbiome. Science has shown a clear correlation between changes in gut bacteria and fatal illnesses like colorectal cancer. Additionally, certain circumstances lead to a dysbiosis of the microbes in the gut. This literature review's primary purpose is to determine the specific gut microbiome related with the progression of colorectal carcinomas, as well as the specific gut bacteria that have demonstrated resistance to various antimicrobial medications. In addition, the review draws attention to the many therapeutic methods that have been identified by rigorous researches conducted.

Keywords: Colorectal Cancer, Drug Resistance, Gut microbiome, Crispr/Cas 9, Nano therapy, phage therapy.

INTRODUCTION
The bacteria, viruses, archaeb, along with other eukaryotic organisms that live in our bodies make up the gut microbiome composition. These bacteria have a huge capacity to influence both the health, wellbeing as well as can bring the onset of ailments in an individual’s physiology. Modern technical advancements have made it possible to undertake sophisticated, culture-independent analyses of the human gut microbiota. In majority of the researches, processes such as 16S rRNA gene analysis is used to identify the microbial component, which is then compared to databases harboring the bacterial sequences. At progressively higher levels of physiology, additional methods such as metagenomic analysis, proteome analysis, transcriptome analysis, metabolome analysis all aid in the analysis and therefore providing more valuable information about the gut microbiome constitution.

Among all the illnesses linked to gut microbial dysbiosis, colorectal cancer is one of the deadliest conditions ever. Colorectal cancer prognosis is determined either as a result of screening or when a patient exhibit symptom. The illness may cause a variety of symptoms, including blood in the faeces, changes in gastrointestinal symptoms, and stomach pain. Other symptoms of anaemia include a pale complexion and shortness of breath, as well as weariness, weight loss, and these symptoms. Even while these symptoms have a limited capacity to predict whether a senior patient would develop colon cancer, further clinical research is still necessary. The commensal microbiota of an individual is present from birth and evolves with time, environment, and dietary intake. Microbiome imbalances have been connected to a range of diseases, including colorectal cancer. The imbalance in the gut microbiota may be caused by a variety of causes, such as dietary changes, new medicines, alcohol consumption, and exposure to alcohol. Prior to now, colon malignancies were quite uncommon, but they now have the moniker "common occurrence," accounting for at least 10% of cancer-related fatalities. Additionally, after gut microbial dysbiosis and the initiation of such tumours, the development of antibiotic resistance among the microbiome population has interfered with the effectiveness of the medications.

This review focuses on the processes promoting dysbiosis as well as the development of colorectal malignancies because of gut microbial dysbiosis. Additionally, it lists the many circumstances in which the dysbiosis of the gut microbiome occurs, along with the resistance towards antibiotic formulations as well as potential therapeutic measures.
GUT MICROBIOME RESPONSIBLE FOR THE INDUCTION OF COLORECTAL CANCER

1. **Fusobacterium nucleatum**

According to a 2018 study by Chen et al., *Fusobacterium nucleatum* reduces the amount of CD4+ T cells in the tissues it inhabits, which causes the pathophysiology of colorectal cancer. These led to a decline in immune responses that are antitumor-mediated, which accelerated the development of colorectal tumours. The research also showed that the amount of another protein called TOX, which is crucial for the differentiation and growth of thymocytes, is decreased in bacterial-infested tissues.

2. **Bacteroides fragilis** (Enterotoxigenic)

According to a 2009 study, this particular gut bacterium generates a toxin that causes inflammation of the epithelial lining, which leads to the development of tumors as well as the onset of related issues including diarrhea. The Wnt pathway is also activated, along with proinflammatory MAPK signaling, as a result of the enhanced cytokine production and increased intestinal permeability caused by the adherence of metalloproteinase toxin (Zn dependent) to the colonic epithelial cells. The intestinal epithelial lining develops carcinomas as a result of this. Furthermore, it was discovered in a different study using mice models that the same particular toxin produced by the bacterium can exacerbate the reactions causing epithelial lining inflammation and tumor induction. The study also made a breakthrough by establishing for the first time that the development of carcinomas is significantly influenced by T helper cells 17, or Interleukin 6, and bacterial toxin. Additionally, through pathogenic inflammatory pathways, this toxin also contributes to the development of colorectal cancer by promoting the differentiation of bone marrow cells into myeloid suppressor cells.

3. **Escherichia coli**

*Escherichia coli*, a member of the intestinal microbiota, has clearly made a significant contribution to the emergence of colorectal cancer. A study found that it enters the mucosal stratum of the intestinal epithelia by processes such DNA damage, interference with DNA repair, acceleration of the mitogenic signaling cascade, and aberrant E Cadherin activity. Together, they contribute to the development of colorectal carcinomas. The involvement of *E. coli* in the carcinogenesis at the colorectal lining was further proved by a study using animal models relating to inflammation as well as xenografts. The study examined and identified that the subtype B2 of the bacterium *E. coli* harbors a unique genetic island called the “polyketide synthase (PSE)”, driving the events of generation of “peptide-polyketide colibactin”. This genotoxin has its proven ability to pass through colonic cell membranes and move to the nucleus, where it disrupts DNA double strands, stops the normal cell cycling, and only partially repairs damaged DNA, leading to chromosomal abnormalities and eventually cancer.

4. **Salmonella typhimurium**

In addition to all the bacterium discussed above, *Salmonella typhimurium* also has substantial contribution towards colorectal carcinogenesis. *Salmonella* sp. are known for their evident ability to release the effector protein “AvrA” to facilitate ubiquitination as well as acetylation of specific proteins of target. “AvrA” prevents the breakdown of beta-catenin, maintains its integrity, and stimulates the proliferation of intestinal epithelium, all of which aid in the development of tumours. “AvrA” also promotes tumour growth and broadens the variety of tumours.

MECHANISM OF INDUCTION OF COLORECTAL CANCERS

Significant amount of research literatures has tried to prove that the formation followed by the progression of colorectal cancer is driven by several processes, much specifically known as inflammatory pathways, mediated by an inflammatory microenvironment. Further researches have established that the microenvironment results in the stimulation of related signaling pathways like Wnt, TGF-beta or the Notch pathways, which in turn affects the self-renewal of the epithelial cells of the mucosal layer at the colon. The release of cytokines and growth factors, as well as the stimulation of transcription factors like NFkB and STAT3, are just a few additional processes that these immune cells oversee, affecting the immunological autoregulation and consequently process of tissue repair at the colon. Additionally, they engage the MAPK pathway (evident in case of infection by *Bacteroides fragilis*), which impacts the ability of cells of the colon to undergo mitosis and thus their survival. The toxin secreted by *Bacteroides fragilis* aids towards the proliferation of T helper cells along with the production of Interleukins such as IL17, IL6, IL17a, etc. Together, all are responsible for the induction of carcinogenesis in the epithelial cell lining of the colon.

Apart from this, other mechanisms such as DNA damage also contributes towards the development of colorectal cancers. The gut microbiota dysbiosis is also related with the induction of DNA damage, producing their own compounds acting as DNA mutagens. For an instance, *Enterococcus* sp. produces certain hydroxyl free radicals that causes DNA strand breaks, along with point mutations. In addition to this, toxins produced by *Bacteroides fragilis* can also bring damages to the DNA strands. “Spermine oxidase” is expressed on the colonic epithelial cells further due to toxins produced by *Bacteroides fragilis*, inducing damages to the DNA. Therefore, such bacterial species contributes towards the pathophysiology of colorectal carcinomas.

It is clear that the colon lining needs a correct growth-death process of cells in order to maintain homeostasis, failing which might result in the establishment of tumours. Any interference with the process of apoptosis (cell death)
might trigger the unchecked growth of additional aberrant cells, which can result in the formation of carcinomas. For instance, the toxin secreted by Bacteroides fragilis can promote E-cadherin breakdown and oncogene transcriptional activation, which results in unchecked cell proliferation at the epithelial lining of the colon and eventually leads to carcinogenesis. In the case of Fusobacterium nucleatum, FadA enhances the bacterium’s interaction with E cadherins, which ultimately results in quite the same fate—uncontrolled cell proliferation and the development of carcinomas—and is another clear example of this pathway of cell proliferation.

**CONDITIONS AT WHICH MICROBIAL DYSBIOSIS OCCURS**

Microbial dysbiosis at the gut can be attributed to a number of different factors. Such factors are listed below:

1. **Diet**

   One’s diet can be attributed the tagline of one of the most important factors controlling the dysbiosis of the gut microbiome. Diets having high fats or high fiber or consisting of high amounts of animal proteins, all contribute to dysbiosis. In a 2010 study to determine the effect of diet in dysbiosis in people by comparing between two groups of people consuming “western diets” and “agrarian diets”, it was found that people associated with the consumption of “western diets” have increased risk of gut microbiota dysbiosis, with an elevated count of Actinobacteria sp., Firmicutes sp. or Proteobacteria. On the other hand, in people consuming “agrarian diets”, an elevated count of Actinobacteria sp. was recorded. In another 2013 study, it was proved that amount of fiber consumed through diet is inversely proportional to the development of colorectal cancers. A high fiber diet can lead to the formation of colorectal carcinomas and vice versa. A bacterium’s ability to produce butyrate has also been linked up with the development of Colorectal cancers. Any decrease in the number of such bacteria can be linked up with the development of cancers at the gut. Thus, having a fiber rich diet elevates the number of specific bacteria producing butyrate, ultimately contributing to the downregulation of the pathogenesis of colorectal cancer. Vitamin D deficiency has also proved its association with the increased in the risk of such cancers by promoting inflammation due to dysfunctioning of the epithelial barrier of the intestine.

2. **Gut Environment**

   In addition to all the above-mentioned factor, another crucial factor which influences the gut microbial dysbiosis is the gut environment itself. Under normal circumstances corresponding to a healthy gut environment, has a lower oxygen concentration, thus harboring a greater obligate anaerobic bacterial population. Furthermore, any increase of oxygenating compounds in the gut environment would result in the disruption of the anaerobic environment, shifting towards aerobic. This shall result in the proliferation in the number of facultative anaerobic bacterial population resulting to dysbiosis.

3. **Genetic reasons**

   Genetic mutations have also resulted in the loss of functionality of the proteins produced by respected genes. For instance, mutations in Interleukin or IL 10 along with IL 10R results in the loss of functionality of the respected proteins. In case of IL 10, mutations can be attributed to factors such as insertion, substitution, deletion progressing towards a stop codon. According to a study, IL 10R mice as well as humans have shown significant defect in the anti-inflammatory signaling cascade mediated by IL 10. This series of events leads to significant inflammation at the intestinal epithelium with a rise in the number of proinflammatory cytokines, and most prominently an upsurge in “TNFα”.

   Innate cells' lack of the IL 10 signal pathway can also affect their ability to communicate with T cells, that worsens the mucosal immunological imbalance and increases the inflammatory responses in the intestine.

4. **Other factors**

   Apart from all these factors, alcohol has a substantial effect in driving the progression towards the development of carcinomas. For instance, it has been demonstrated that the proportion of Enterococcus faecalis in those with Alcoholic Hepatitis has significantly increased. The discovery that alcohol enhanced the permeability of gut cell membranes led to the transfer of “Cytolisin”, a bacterial exotoxin, to the liver cells and caused damage to the liver cells was a big step forward. As a result, drinking alcohol causes alcoholic hepatitis in people.

**EMERGENCE OF DRUG RESISTANCE IN GUT MICROBIOME**

Antimicrobials revolutionized medical practice; however, their efficiency has been weakened ever since they were first identified due to the rise of antibiotic resistance. On antibiotic target mutations or antibiotic resistance genes (ARGs), antimicrobial resistance can be encoded. Mobile genetic elements (MGEs) and extrachromosomal plasmids can be used to transmit these changes horizontally and vertically through microbial populations, respectively.

Gut, the most densely populated microbial environment, serves as an important reservoir for antimicrobial resistance (AR) organisms. The host receives significant advantages from the stable, diversified population of the healthy gut microbiome, including nutrition absorption and pathogen defence. The high microorganism population in this habitat makes it easier for pathogens to acquire antimicrobial resistance (AMR) genes by horizontal gene transfer (HGT). Since, gram-negative bacteria include extended-spectrum beta-lactamases (ESBLs), particularly Enterobacteriaceae, have acquired several genes of resistance during the past few years and are frequently resistant to third-generation cephalosporins. Escherichia coli, a microorganism that dwells in the human gut and often gets along well with the host, has become more
frequent as a result of the development of this resistance mechanism that first appeared in *Klebsiella pneumoniae* 32.

By altering the taxonomic and functional makeup of this ecosystem, antibiotics might cause disruption and open up new entrances for pathogens 33. For instance, some antibiotics, such as the often-recommended tetracycline and macrolides, can destroy beneficial gut flora, cause gastrointestinal complications, and result in urinary infections. This “dysbiosis” may facilitate the colonization of AR, a rise in ARG load, and eventual invasion of the AR pathogen into the blood, urinary tract, and other organ systems 34.

**Figure 1: Mechanism of bacterial colonization resistance**

The colon often interacts with a variety of bacteria. Proteobacteria and Fusobacteria, which make up the majority of the colon microbiota including *Enterococcus faecalis*, *Escherichia coli*, *Streptococcus gallolyticus*, *Bacteroides* sp., and *Clostridium septium* seem to be most commonly correlated with the onset of colon cancer 35, may raise the risk of colorectal cancer (CRC) by generating toxic metabolites or having direct impacts. The third most prevalent disease in both men and women globally is colorectal cancer (CRC). Surgery, targeted therapy, neoadjuvant radiation, and adjuvant chemotherapy comprise the foundation of CRC treatment. However, while patients with stage I CRC have a 90% chance of survival, those with stage IV CRC have just a 10% chance. One of the concerns with the low survival rates of CRC patients continues to be drug resistance. For the development of new drugs, a greater knowledge of inherent and acquired therapeutic resistance would be immensely significant 35.

**RESISTANCE SHOWN BY MICROBIOME TOWARDS CERTAIN DRUGS**

1. **5 fluorouracil (5-FU)**

Five-fluorouracil (5-FU) is a pyrimidine analogue that is used to treat colorectal cancer. It belongs to the family of antimetabolites. The primary ingredient is capecitabine’s active metabolite. The cytotoxic medicine 5-FU inhibits cancer cell growth by binding to and destroying the thymidylate synthase enzyme. This results in DNA double strand breaks as well as RNA, cell cycle arrest, and eventually cell death 36,37.

The presence of *F. nucleatum* has been associated to resistance to a tegafur and oxaliplatin chemotherapy combination in individuals with colon cancer 38. It has been found that infection with *Fusobacterium nucleatum* is linked to resistance of colorectal cancer to “5-fluorouracil (5-FU)” through boosting the expression of the baculoviral IAP repeat containing 3 (BIRC3) protein. The immunological host system is weakened by *F. nucleatum* because of its interactions with immune cells, which increase tumor-associated neutrophils, pro-cancer M2 macrophages, and dendritic cells, while concurrently suppressing the cytotoxicity of T and NK cells 39.

In several studies, it was shown that the efficacy of fluoropyrimidines was diminished in the presence of mycoplasma species or bacteria 40. Mycoplasma hyorhinis infection of cell lines led to a direct reduction in the tumour cells’ sensitivity to pyrimidine nucleoside analogues. The potential anticancer action of “5-fluoro-2-deoxyuridine” and “trifluorothymidine” is significantly reduced (20-150-fold) upon degradation to the less effective base, SFU, or the inert trifluorothymine, respectively 40.

2. **Oxaliplatin**

Traditional chemotherapy for gastrointestinal malignancies, such as the “FOLFLOX” (colorectal cancer) and “FOLFIRINOX” (pancreatic cancer) combination regimes, employ a platinum derivative called oxaliplatin 41. It causes cells to undergo apoptosis by blocking their ability to replica their genetic material (RNA, DNA, and proteins). It is possible to kill cancer cells by preventing their proliferation by a combination of DNA damage, halting DNA synthesis, suppressing RNA synthesis, and activating the immune system 42. Oxaliplatin’s unique chemical structure, with a “1,2-diaminocyclohexane (DACH) ligand”, increases its potency against tumour cells by making DNA repair more difficult.

In addition to downregulating microRNAs (miR-18a as well as miR-4802), *F. nucleatum* has been demonstrated to activate autophagy, leading to resistance to SFU and oxaliplatin in cell culture 43. Due to its evident activation of the innate immune system, *F. nucleatum* is also primarily responsible for the chemoresistance to 5-FU and oxaliplatin in patients with colorectal cancer 44-46.

The effectiveness of oxaliplatin was significantly diminished when the microbiota was eradicated by antibiotics. In germ-free mice, a comparable decline in effectiveness brought on by the lack of resident flora was also detected 46. Oxaliplatin resistance is associated with the nucleotide excision repair nucleotide excision repair (NER) pathway and the WBSR22 protein, which are biomarkers for the expression of ERCC1, and XDP, and XRCC1 47.

3. **Irinotecan**

Irinotecan, a semisynthetic version of camptothecin that acts as a topoisomerase-1 inhibitor, is commonly used in combination with other anticancer drugs to treat colorectal cancer (FOLFIRI). The FDA approved the chemotherapy agent irinotecan (CPT-11) for the treatment and prevention of CRC in 1996. “7-ethyl-10-hydroxycaimtophecin (SN-38)”, which has a thousand-fold greater anticancer action than CPT-11, is produced when “CPT-11” undergoes intracellular modifications in the cell,
such as the removal of the C10 group via carboxylesterase catalysis 48.

Low intratumor levels of the active metabolite “SN-38”, decreased “topoisomerase I” expression, altered activity of the “SN-38-Topo I-DNA complex”, and alterations to subsequent events, such as the suppression of apoptosis, cell cycle changes, or increased DNA repair, all appear to contribute to irinotecan resistance in CRC (49). “CPT-11” based chemotherapy induced microbial dysbiosis in the gut by favouring potentially harmful bacteria like Enterobacteriaceae as well as Clostridium spp. but rather reducing beneficial bacteria like Bifidobacterium spp & Lactobacillus spp. 49.

It is believed that the negative effects of “CPT-11” are the result of the inactive “SN-38 G” being converted back to the active and toxic “SN-38” by microbial glucuronidases (GUDS) in the intestines 50. The GUDS of commensal bacteria, for instance Bifidobacterium spp., is less active in the “SN-38” conversion than the GUDS of Enterobacteria and other opportunistic bacteria, for example Clostridium spp. or Escherichia coli, due to the presence of different chemicals 51.

4. Capecitabine

Capecitabine, the first oral chemotherapeutic medication for CRC, is used in a variety of settings, depending on the patient’s needs: as adjuvant therapy, as monotherapy, or in combination with other medications for advanced or metastatic disease (52). Capecitabine inhibits tumour development and metastasis by destroying tumour cells or stopping their division and spread 52. “5’-deoxy-5’-fluorocytidine (5’-FOCR)” and “5’-deoxy-5’-fluorouridine (5’-DFUR)” are two by-products of this substance's breakdown in the body. After then, thymidine phosphorylase (TP) converts “5’-DFUR” to “5’-FU”, which then has a cytotoxic effect 52,53.

There are many common resistance mechanisms in “5’-FU” resistance. Its resistance is primarily mediated by TP, an enzyme required for the conversion of capecitabine to “5’-FU”. Over express of TP will react favourably to capecitabine, whereas having lack of function develops resistance 53.

5. Anthracyclines

Produced by Streptomyces strains, anthracyclines have a bacteriostatic effect that slows the proliferation of cancer cells by intercalating between the base pairs of DNA (or RNA). Doxorubicin (DOX) is an excellent adjuvant chemotherapy drug for advanced CRC 53.

Some bacterial species are capable of breaking down anthracyclines. Streptomyces WAC04685 has the ability to de glycosylate DOX, rendering it inactive (53). Raoultella planticola, another gut bacterium, may use the same process to de glycosylate doxorubicin to such compounds as “7-deoxydoxorubicinol” and “7-deoxydoxorubicinone” 54,55.

It is hypothesised that DOX-induced increased Reactive oxygen species contribute to CRC’s resistance to chemotherapeutic medications by activation of drug transporter proteins, multi-drug resistance proteins, as well as genetic/epigenetic changes (ROS)53.

Table 1. List of different bacteria showing resistance to specific drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Resistance bacteria</th>
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<tbody>
<tr>
<td>5-fluorouracil (5-FU) 38,39</td>
<td>M. hyorhinis</td>
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<tr>
<td></td>
<td>F. nucleatum</td>
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<tr>
<td>Oxaliplatin</td>
<td>Commensal bacteria</td>
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<tr>
<td>Irinotecan 49,50</td>
<td>Lactobacillus</td>
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<td></td>
<td>Bifidobacterium</td>
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<td>Staphylococcus</td>
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<td></td>
<td>Clostridium</td>
</tr>
<tr>
<td>Anthracyclines53-55</td>
<td>Streptomyces WAC04685</td>
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THERAPEUTIC INTERVENTIONS FOR OVERCOMING DRUG RESISTANCE IN COLORECTAL CANCER

Drug resistance have emerged as a major barrier in treatment of colorectal carcinomas.

1. Crispr/Cas 9

Crispr Cas 9 has spread its arm welcoming the treatment of such carcinomas using this technique. The system acts like an immune system based on RNA. This system can be used to edit genes such as MDR genes in a system. It requires a single guide RNA known as sgRNA for the effective entry of the target gene of interest along with Cas 9. The single guide RNA can guide Cas9 up to the target sequence, where Cas 9 facilitates cleaving the cleavage of the double stranded molecule, thereby facilitating insertion or deletion 56. Doxorubicin, a crucial and affordable medication, is used to treat a variety of carcinomas, including colorectal cancer 57. According to a study on the cancer cells “HCT-8/V” and “KBV20”, it was clear that employing Crispr Cas 9 to delete the “ABCB 1” gene resulted in a considerable buildup of doxorubicin inside the cells and thus increased the sensitivity to chemotherapy 58. The delivery of genetic material (mRNA or plasmid DNA) coupled with sgRNA as well as Cas9 or delivering Cas9 and sgRNA RNP complexes are the two methods that CRISPR/Cas9 components can be supplied by employing the use of lipid nanoparticles. The technique works analogous to microinjection when Cas9 mRNA/sgRNA are employed 59. But numerous study groups have found that Cas9:sgRNA RNP complexes are incredibly effective 60,61. Numerous research has used CRISPR-Cas delivery techniques using nanoparticles or liposomes to successfully halt the growth of biofilms which promotes drug resistance.
2. Inhibitor mediated

It was established in a different study that "ATP binding cassette superfamily G member 2 (ABCG2)" provides drug resistance abilities against a number of chemotherapeutic medicines. The development of new "ABCG2" inhibitors, such as "Ataxia-telangiectasia mutated kinase inhibitor AZ32," which could sensitize "ABCG2" over expressing cells of colorectal carcinomas to chemotherapeutic drugs like mitoxantrone and doxorubicin by remaining inside the cells, provided a practical solution to this medication resistance over time (62). Accordingly, the study found that "AZ32" has the capacity to successfully combat the phenomena of drug resistance mechanisms made possible by "ABCG2" (62).

3. Nano therapy

Today, the biggest barrier to cancer chemotherapy is the emergence of drug resistance. The use of nanomaterials for the efficient delivery of drugs has its evidence to be a promising technology for controlling drug resistance (63). According to a couple of 2021 studies by Xue et al. and Wang et al., it has been revealed that clinical resistance to chemotherapy due to molecular alterations or signal transduction pathways can be attributed to the phenomena of "hypoxia" (64,65). The ways of controlling hypoxia by employing the use of nanomaterials have been researched, can be split into three major categories comprising, combating hypoxia, neglecting hypoxia or manipulating hypoxia. The strategy of combating hypoxia is the most common approach of targeting. For instance, Acetazolamide loaded in a flexible nanoparticle directed to tumour hypoxia, was developed in a recent study with the goal of overcoming Sorafenib drug resistance by targeting the tumour hypoxia marker "carbonic anhydrate IX" (66).

Another study uses OCD nanoparticles to transport the anticancer medication camptothecin-11 to the intestine's cancerous areas. According to the study, neither the pH levels in the intestines nor the presence of a lot of reactive oxygen species could prevent the medicine from being released from the nanoparticles. This method demonstrated strong anti-tumour properties (67). The development of a liposome nanodrug combining glucose oxidase, platinum therapeutic agent, and tirapazamine is an unique nano-chemotherapy technique that has just been presented as a therapeutic intervention for colorectal carcinoma (68).

4. Expression of apoptosis

Since apoptosis is suppressed in drug-resistant cancer cells, restoration of apoptotic signals and inhibition of cancer cell growth by alternative cell death pathways are proposed to be effective means to treat such resistant cancers.

The phenomenon of routine cell death (apoptosis) is suppressed in cancer cells resistant to drugs, it is suggested that restoring the signals of apoptosis and preventing cancer cells proliferation by using alternate pathways of cell death are viable strategies to treat such resistant tumours, ultimately suppressing the pathology of such carcinomas. BH3 mimetics, tiny compounds that mimic BH3 only proteins by putting their BH3 specific domains into the “hydrophobic grove” of Bcl2 proteins and promote apoptosis in order to repair the defective apoptotic signals inside the cancer cells (69). For instance, ABT 263, a BH3 mimic, and carfilzomib-an anticancer drug, cooperatively increased apoptosis in carcinoma in carcinoma cells that were resistant to apoptotic cell death due to mutation in KRAS gene (70).

5. Using Bacterial Viruses

The bacterial viruses known as bacteriophages have the innate ability to infect exclusively a certain bacterial community. As a result, bacteriophages are used to alter the gut microbiome that causes colorectal cancer. To deliver the phages to the site of the intestinal cancers, they are enclosed in microspheres. This review has previously listed the various gut microbiome such as Fusobacterium nucleatum, Enterococcus faecalis, Bacteroides fragilis, Salmonella typhimurium, all are associated with the induction of colorectal cancer. Typically, bacteriophages specific to the gut flora include phage VA7, M13 and phage T7, ZCECS, T4 along with phage UAB Phi 20, UAB Phi 78, UAB Phi 87 specific to Bacteroides fragilis, Fusobacterium nucleatum, Escherichia coli as well as Salmonella typhimurium respectively. However, the most difficult part of employing this method is ensuring that the microspheres are properly packaged such that there is a little loss in phage titer upon initial contact with the acidic environment of the stomach (71,72). Numerous new techniques for creating stable microspheres have been discovered with the passage of time and thorough investigation throughout the years. For instance, sodium alginate was combined with honey or gelatine in a 2019 study by Abdelsattar et al. to encapsulate the phage ZCECS that was intended to target Bacteroides fragilis (71). The identical phage, phage ZCECS, was previously delivered using chitosan or polyethyleneimine coating of sodium alginate beads for the purpose of targeting Bacteroides fragilis in a 2017 study (72).

FUTURE APPLICATION

Multiple novel pathways of antibiotic resistance in the gut microbiota and promising treatment approaches have been discovered. Nonetheless, there are a number of topics that may benefit from more study. We now know that bacteria may exchange drug-resistant genes with one another through processes called horizontal gene transfer (73–75). It is unusual for the whole flip of drug-resistance genes to occur because of stressors contacting the genetic components during the transfer and damaging the DNA (76,77). Therefore, further in-depth investigations are required to comprehend how genes are transported throughout microbiome communities. Additionally, such microorganisms are capable of biofilm development, and the vast majority of resistance gene transfer occurs in biofilms by conjugation or transduction (76,78,79). Therefore, it is
important to focus on dismantling bacterial biofilms to limit the dissemination of drug-resistance genes. The gut bacteria are also in charge of keeping the digestive system in balance. When several gut bacteria are infected at once by a phage, additional bacteria that do not cause colorectal cancer may acquire the phage’s virulence components and grow into a disease. For phages that infect many species of gut bacteria, it is important to do a risk-benefit analysis to identify any possible concerns and develop strategies for mitigating them.

**CONCLUSION**

Several illnesses, including colorectal malignancies, have been linked to the gut microbiota. The emergence of medication resistance is the primary concern related to gut microbial dysbiosis. Even when bactericidal medications were present, the germs still multiplied because they were resistant to those treatments. Several therapeutic treatments have been found as a strategy to combat medication resistance in the gut microbiota, thanks to the efforts of scientific researchers motivated by a desire to find a solution to the most commonly discussed problem prevalent in modern society. Phage treatment, Crispr/Cas 9, nano therapy, and other recently discovered methods have all shown promise in the fight against drug resistance. Studies conducted in recent years aimed at verifying the efficacy of such therapeutic intervention have led to its incorporation into standard medical practice. However, additional investigations need to be formed to tackle the rising challenges linked with such therapeutic methods, so that more emerging outcomes may be established in the battle against multi-drug resistance.

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**REFERENCES**


