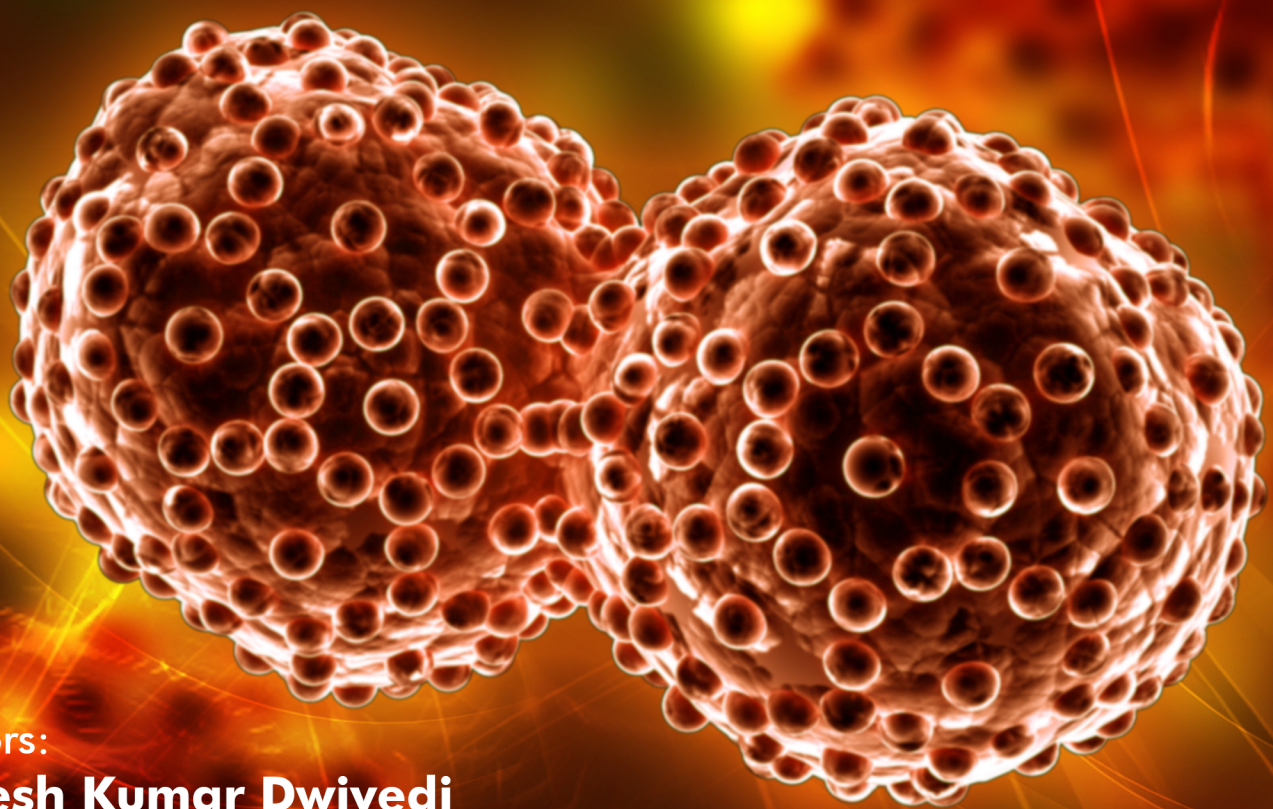
Probiotics in Anticancer Immunity



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Frontiers in Cancer Immunology

(Volume 3)

Probiotics in Anticancer Immunity

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CONTENTS

FOREWORD

Nowadays, the probiotics field (a scientist that investigates; industry, production process; regulators, and protection to consumers, and consumers) is an interesting topic in the nutritional field that is constantly being discussed (medical, immunological) related to human health. Who thought that 100 years ago, when Russian immunologist Melnikoff noted that Bulgarian farmers are healthier and live longer? This Nobel Prize winner supposed that this is because the fermented milk they use contains live bacteria, which gave rise to the modern concept of probiotics, "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host.

As a researcher in the probiotic field, it is always good to reinforce the knowledge and see how much we know about probiotics, and how they could help us in the prevention of children's diarrhoea, cases of allergy, or a coadjutant treatment in immunological disorders or even cancer.

Today I have the honour to introduce the volume 3 of book titled "Frontiers in Cancer Immunology [Probiotics in Anticancer Immunity]", which include a total of 11 chapters with 283 pages and edited by Dr. Mitesh Kumar Dwivedi, Dr. Alwarappan Sankaranarayanan, and Dr. Sanjay Tiwari.

The book is focused on the mechanical characteristics that probiotics generate in several human cancers, with evidence-based medicine based on human studies and its effects along with the comprehension of key molecules of cancer progression using animal studies. In my personal experience, knowing how probiotics work, we obtained a better general vision of their effects in different conditions. An expert team of researchers worked on the compilation of the book with a special final chapter related to "Future Challenges in Probiotics Based Anticancer Immunotherapy", which mention the need for more human clinical trials with a higher number of subjects that allow us to understand the correct dose/benefit of the use of probiotics as a coadjutant therapy.

I think that the present book will have an impact on the probiotics field because the topic is related to our clinical research; now, we will be investigating how microbes are present on the surface of different mucosa and how they could affect the progression of the disease or the treatment.

Hopefully, more investigation in the probiotic field will be made in the next years to understand the true mechanism of action of the probiotics in different human conditions.

All the best with the present book.

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PREFACE

The book 'Frontiers in Cancer Immunology (volume 3) Probiotics in Anticancer Immunity' is focused on the role of probiotics in exerting the anticancer immunity. With the diverse role of probiotics in benefitting the human health, their role in prevention and management of various human cancers cannot be denied. The book is focused on delivering the evidence of the use of probiotics in human cancers through several animal and human studies, in addition to highlighting their mechanistic role.

The book 'Probiotics in Anticancer Immunity' consists of total 11 chapters. The initial two chapters provide the basic background of the interaction of gut microbiota and host immune system in cancer and the different mechanisms by which probiotics can induce/exert the anticancer immunity.

The subsequent chapters deal with the specific cancer conditions such as cancer of skin, colon, colorectal, breast, stomach, liver, cervical, lung, and head & neck, and mechanistic role of probiotics in inducing the anticancer immunity. Moreover, the role of gut microbiota in the dysbiosis and management and/or prevention of these cancers is also put forward.

We, the editorial team, strongly believe that the contents of the individual chapters will provide recent and updated information as well as new insights into the role of probiotics in anticancer immunity. As such, the book will be useful in education and as a scientific tool for academics, clinicians, scientists, researchers, and health professionals in various disciplines including microbiology, medical microbiology, immunology, biotechnology, and medicine.

As the editors, we would like to express our sincere gratitude to all authors for their excellent contributions. We are also indebted to the publishers for their efforts to publish the book in a timely fashion.

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CHAPTER 1

Gut Microbiota and Host Immune System in Cancer

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Abstract: The mammalian gut is inhabited by more than 100 billion symbiotic microorganisms. The microbial colony residing in the host is recognised as microbiota. One of the critical functions of microbiota is to prevent the intestine against exogenous and harmful pathogen colonization mediated by various mechanistic pathways involving direct competition for limited nutrients and regulation of host immunity. Cancer accounts for one of the leading causes of mortality arising from multifactorial abnormalities. The interconnection of microbiota with various pathological conditions including cancer is recently being researched extensively for analysing tumor induction, progression, inhibition and diagnosis. The diversified microbial colony inhabiting the human gut possesses a vast and distinct metabolic repertoire complementary to the mammalian enzyme activity in the liver as well as gut mucosa which facilitates processes essential for host digestion. Gut microbiota is often considered the critical contributor to defining the biochemical profile of diet thus impacting the health and disease of the hosts. This chapter mainly focuses on understanding the complex microbial interaction with cancer either negatively or positively which may help to conceive novel precautionary and therapeutic strategies to fight cancer.

Keywords: Adenocarcinoma, Cancer, Carcinogenesis, Dysbiosis, Dysplasia, Gut microbiota, Hyperplasia, Homeostasis, Inflammatory pathways, Metaplasia, Metabolism, Metabolomics, Metagenomics, Oncogenes, Pathogens, Tumorigenesis.

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Mitesh Kumar Dwivedi, Alwarappan Sankaranarayanan & Sanjay Tiwari (Eds.)

1. INTRODUCTION

The gut microbiota of humans is recognized as a complex and dynamic heterogonous ecosystem which is comprised of diverse microbial communities such as bacteria, archaea, viruses, fungi, *etc*. interacting with each other and also with the host. All the genes of microorganisms taken together build a genetic repertoire representing an order of higher magnitude than that of humans. Being the most extensive micro-ecosystem existing in human body, it is considered an essential organ. Its symbiotic nature with host's body allows it to play a major role in regulating the various physiological processes. Gut microbiota is mainly categorized in four major sections namely *Firmicutes, Proteus, Bacteroides* and *actinomycetes*. The complex and cross-linked adaptive and innate immune system play a pivotal role in maintaining the homeostasis of host defence system against harmful pathogens. With the rapid advancement in molecular biology, bioinformatics, genomics, analysis technology *etc*. gut microbiota research has made immense progress. Such research has pointed out that compromised gut microbiota and their metabolites often contribute to pathological developments such as neurodegenerative problems, metabolic and gastrointestinal disorder and even cardiovascular diseases. Current evidence also reflects the involvement of microbiota in carcinogenesis and may improve the activity and efficacy of anticancer therapies or might also increase their toxicities in contrast. In this chapter we have summarized the relevance of gut microbial alteration with various cancers and also discussed the association of probable metabolic mechanisms of microbes and their derivatives with the development of cancer and also their facets of anti-tumorigenic properties. Thus, the present chapter reflects the link of gut microbiota with the host immune system and its role in the modulation of carcinogenesis.

2. HUMAN HEALTH AND DISEASE: ROLE OF GUT MICROBIOTA

More than 1000 million symbiotic microorganisms live inside human beings and exert a significant role in human health and disease. The gut microbiome has been considered a "fundamental organ" [1], containing about 150 times more genes than that of expressed in the entire human genome sequence [2]. Recent advancement in research has revealed that the microbiome is implicated in basic biological activities of human being, influencing innate immunity, regulating development of epithelium and modulation of metabolic phenotype [3 - 6]. Several chronic ailments like IBD, ulcerative colitis, obesity, metabolic disorder, atherosclerosis, ALD, NAFLD, liver cirrhosis, as well as hepatocellular cancer have been related through the human microbiome [7, 8]. In current decades, a remarkable extent of evidence has intensely recommended an important function

of the gut microbiome in health and disease of humans being [9 - 23] *via* numerous mechanisms of actions. Significantly, gut microbiota has the ability to upsurge extraction of energy from nutrient, enhance nutrient production and modify signalling of appetite [9, 10]. Gut microbiota has additional multipurpose metabolic genes as compared to the genome of humans, and delivers individuals with specific and distinctive enzymes and several biological and chemical pathways [9]. Firstly, an immense quantity of the metabolic gut microbiota use that are advantageous towards the host are concerned in both acquirement of dietary or xenobiotic dispensation, comprising with the metabolic process of undigested biological compound and the vitamins production [10]. Secondly, the gut microbiota of human also delivers a bodily blockade, defending its host against external infectious agents through production of antimicrobial agents as well as competitive rejection [11 - 13]. In conclusion, gut microbiota shows a very important function in the expansion of the abdominal mucosa as well as immunity of the host [14 - 16].

2.1. The Human Microbiome in Health

The human microbiome has a significant impact on host physiology. Bacteria, viruses and trillions of other microorganisms colonize the body of human being, and the gut microbiota are directly associated with host physiology. While more than 1000 known bacterial species dwell in the body, innumerable other microbial genes have been identified in the human genome [2]. After birth, the mutualistic bacteria start colonizing in the host and subsequently evolve into a diversified ecosystem as the host body grows and develops [24]. Symbiotic bacteria assist in metabolism of indigestible food, supplement with requisite nutrients, extend protection against other pathogenic colonization and also play pivotal role in forming intestinal architecture [25]. Thus host-bacteria interrelation has evolved to be beneficial. The intestinal microbiota is involved in maintaining energy homeostasis and often facilitates the digestion of indigestible dietary fibres present in vegetables. While specific *Bacteroides* species help in digestion of xyloglucans present in vegetables, beneficial microorganisms like *Lactobacillus* and *Bifidobacterium* utilize fructo-oligosaccharides and oligosaccharides which are difficult for digestion by host [26, 27]. Moreover, earlier reports indicate that gut microbiota essentially participate in maintaining lipid and protein homeostasis and are involved in synthesis of microbial nutrients like vitamins [28]. Each day, 50 to 100 mmol/L of SCFAs produced by gut microbiota, including acetic acid, butyric and propionic acids, supply energy to the host intestine [29]. These easily absorbable SCFAs in the colon perform diversified roles and regulate motility of gut, inflammatory response, and metabolism of glucose and energy management [30, 31]. Additionally, the gut microbiota is also associated with the supply of

CHAPTER 2

Mechanism of Probiotic Action in Anticancer Immunity

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Abstract: Gut microbiota plays a significant role in human physiology which includes metabolism, nutrition uptake and immune responses. The imbalance of gut microbiota leads to various disorders or diseases like inflammatory bowel disease, infectious diseases, cancer and obesity. Cancer is one of the major health problems worldwide. Moreover, colorectal cancer (CRC) is the most common cancer in humans which is considered the fourth leading health problem worldwide. The role of probiotics in the regulation of CRC includes enhancement of immune barrier function, intestinal immune state, inhibition of enzymatic activity, cell proliferation and apoptosis, redox homeostasis, and modifying the composition of intestinal microbiota. It can be treated using chemotherapy, radiotherapy, or surgical eradication. However, these treatments may cause the demolition of the intestinal mucosal barrier system as well as dysfunction of the immune system in cancer patients. Hence, biotherapeutic drugs are used along with probiotics and their metabolites *viz*. polysaccharides, short-chain fatty acids, and inhibitory compounds like proteins and other substances to treat cancer. *Lactobacillus rhamnosus* GG (LGG) is a widely used probiotic strain in oncology. Also, it has been proven to exert beneficial effects on cancer patients after anticancer therapy. Therapeutic potential of the gut microbiome in cancer treatment *via* the administration of probiotic supplementations is being investigated using several clinical studies. Probiotic-incorporated biotheraupetic drugs are considered an alternative medicine for various types of cancer. The effectiveness of biotheraupetic drugs mainly depends on the dosage of probiotic strain and their exposure time. However, the mechanism behind the role of probiotics in cancer immunity is unclear so far. The present work summarizes the action of probiotics in anticancer immunity.

Keywords: Biotheraupetics, Cancer, Chemotherapy, Gut microbiome, Immunity, Probiotics.

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Mitesh Kumar Dwivedi, Alwarappan Sankaranarayanan & Sanjay Tiwari (Eds.)

1. INTRODUCTION

The gut microbiota is considered one of the most significant factors in maintaining homeostasis. Bacterial strains are involved in the detection and degradation of possible carcinogens,the production of immune system signalling molecules like short-chain fatty acids (SCFAs), which influence cell death and proliferation during carcinogenesis [1]. Due to their capacity to affect cancer cell proliferation and death, probiotics have gotten a lot of attention from consumers. These potential properties are helpful to treat cancer through innovative therapies, which are considered an alternative method to more invasive treatments like chemotherapy or radiotherapy [2]. Several studies have indicated that dysbiosis of the intestinal microbiota contributes to the development of metabolic and intestinal diseases, including obesity, diabetes, and cancer [3]. The microbiota can be tumorigenic or tumor-suppressive, and its regulation and maintenance are critical for the host's overall health. Consumption of probiotics modulates the microbiota, maintains a symbiotic relationship with the host, and ensures the optimal development of the immune system and the efficacy of cancer treatment [4 - 6]. In this chapter, the cancer immunity achieved by probiotic strains is discussed with their mechanism of action.

1.1. Gut Microbiome

The human microbiome is made up of a range of microorganisms that live on the surface of our body's epithelial barrier, including bacteria, fungi, archaea, protozoa, and viruses. The gut microbiome can modulate the host’s immune system to maintain the host’s health. Probiotic bacteria have been proven to have an important function in immunomodulation and have antitumor effects in humans. Also, probiotics have the potential to boost and reduce the production of cytokines which are anti-inflammatory molecules and play a vital role in carcinogenesis prevention. Lactic acid bacteria in the gut have been demonstrated to play a role in carcinogenesis regression by producing metabolites that interact with immunological and epithelial cells due to their influence on immunomodulation [1]. Multiple physiological functions can be affected by the microbiota, especially metabolism, inflammation and immunity. The gut microbiota collaborates with epithelial and stromal cells to perform a variety of important regulatory activities. It maintains mucosal immunological homeostasis and host-microbial symbiosis, as well as prevents pathogen infection [7]. Microbial components in the intestine have a significant impact on the peripheral immune system, especially in the case of cancer [8].

An effect of the microbiota on host bile acids was reported as a carcinogenic driver in some of the studies. Primary bile acids are created in the liver and

released into the small intestine, where bacteria convert them to deconjugated, secondary and tertiary bile acid moieties, which are then reabsorbed by the intestine and transported to the liver *via* portal circulation. Deoxycholic acid (DCA), a secondary bile acid product, is particularly carcinogenic and leads to DNA damage in hepatocytes [9].

1.2. Current Status of Cancer and Treatments

Cancer is the world's second-largest cause of death. In the United States alone, around 18,98,160 persons were diagnosed with cancer in 2021, with 6,08,570 of them dying due to the disease [10]. In India, 13,24,413 people were affected, and 8,51,678 people died of cancer [11]. Thus, cancer is a major issue that has an impact on the health of all human communities. Colorectal cancer (CRC) is the third most commonly diagnosed malignancy and the fourth leading cause of cancer-related death in the world. The treatment for colon cancer is illustrated in Fig. (**1**).

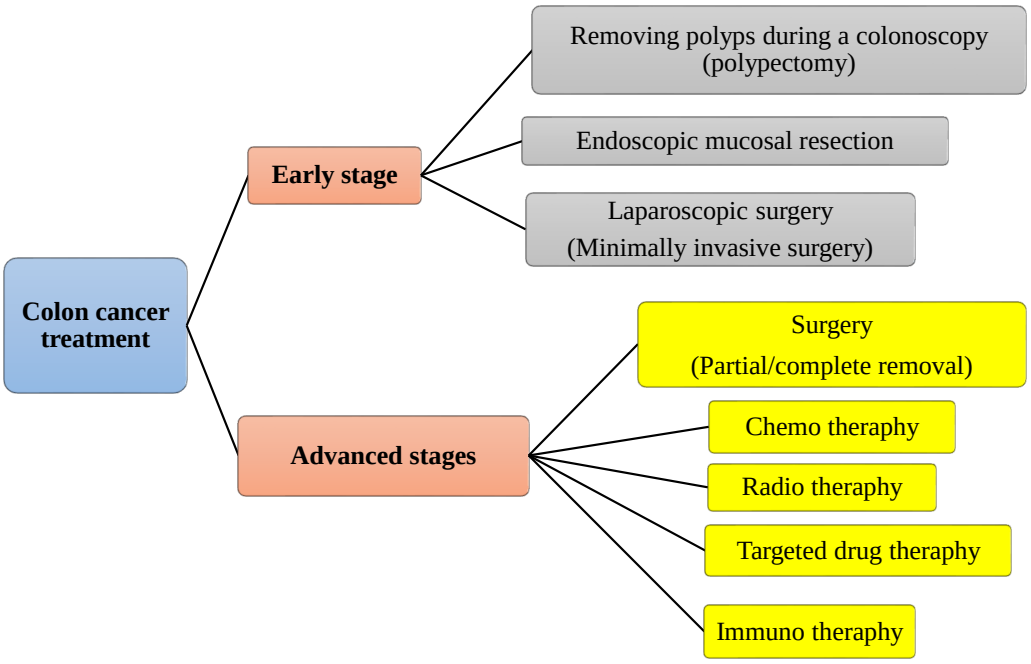

Fig. (1). Treatment strategies for Colon cancer.

Cancer immunotherapy is becoming more popular as a treatment option for cancer patients. It acts as an anti-tumor agent by utilising the immune system. As

CHAPTER 3

Probiotics Based Anticancer Immunity in Skin Cancer

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Abstract: Cancer, a condition caused by unregulated cell proliferation, has elevated the global mortality rate that was rising on a daily basis. The treatments for cancer have numerous adverse effects on patients' lives. To enhance this treatment, probiotics and their metabolites (postbiotics) play an important role in the prevention and treatment of cancer. The mechanisms behind probiotic anti-tumor and/or anti-cancer actions are not yet comprehended. Numerous studies demonstrate that probiotics are useful in cancer prevention and treatment. The majority of which are involved in balancing microbiota, producing essential compounds containing beneficial effects and anti-tumor and cancer activity, preventing pathogen infection, modulating the host immunity, reducing inflammation, and in alleviating the severity of some risk factors. Few studies advise that they should not be used, emphasizing the risk of infection to patients. This chapter provides an overview of skin cancer, skin microbiome, gut microbiome, and its implications in skin cancer, as well as probiotic and postbiotic therapeutic approaches.

Keywords: Lactic acid bacteria, Microbiome, Probiotics, Postbiotics, Skin cancer.

1. INTRODUCTION

Cancer, a condition caused by unregulated cell proliferation, affects millions of people each year and is recognized as one of humanity's worst major health problems [1]. As a result, scientific researchers are aiming to develop a wide range of cancer therapies nowadays. In recent years, there has been a major surge in the investigation of the use of probiotics as an adjuvant to conventional cancer therapies [2]. FAO/WHO defines probiotics as "living microorganisms that provide health advantages on the host when administered in suitable doses." [3, 4]. Probiotics confer benefits in numerous ways, including dropping gut pH,

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preventing pathogen colonization, and modulating the host immune response [5]. Probiotics also prevent and treat cancer by controlling the intestinal microbiota, strengthening the colon, boosting the function of the intestinal barrier, regulating enzymes and metabolisms of gut bacteria, and blocking carcinogenic compounds, producing essential compounds containing anti-proliferative activity, and reducing inflammation [6, 7]. Therefore, probiotics could be a promising tool for skin cancer therapy. However, further study about how to supplement these probiotics and their appropriate dosage is required [8].

2. SKIN CANCERS

Based on the world's earliest records, cancer has been a serious health concern since 3,000 B.C., and its incidence continues to climb to this day [9]. Due to the multiple physiological, social, material, and spiritual threats it poses, cancer is difficult to treat. Globally, around one million new cases of cancer are diagnosed each year [10]. It arises as a consequence of the uncontrolled growth of abnormal cells (neoplasia) in any region of the body. As these cells proliferate, they produce a tumor-like mass [1]. In fact, invasion, metastasis, and uncontrolled growth must be present in the cell in order to speak of cancer [10]. If a normal cell acquires these malign features, it appears to reach the subsequent stage of carcinogenesis. These four phases are as follows: Phase 1 (initiation, progressing extremely gradually, also recognized as the latent stage), Phase 2 (promotion, rapidly growing cell groups, promoters are impactful), Phase 3 (progression, operating independently of the surrounding tissue, genetic mutations are irreversible), and Stage 4 (invasion and metastasis, complicated to treat) [1]. Interfering with cells in the beginning stages of mutation may prevent the development of cancer. Therefore, a clinical diagnosis devoid of risk factors is necessary for each patient. Individuals having a family history of cancer are regarded to be more susceptible [9]. There are currently efforts to create treatment options for some types of cancer. It is well acknowledged that cancer is a disease that varies from person to person. Chemotherapy, radiation therapy, and medical techniques are frequently employed in cancer treatment and are the key factors. Nevertheless, the previously described therapies might have a negative impact on one's quality of life [1]. Cancer, a condition defined by uncontrolled cell proliferation, kills millions of people each year and is one of humanity's most serious health issues [10]. As a result, various researchers are continually working to develop multiple cancer therapy options.

Cancer of the skin is the most prevalent type of human skin. More than 4 million cases of non-melanoma (*i.e.*, Basal Cell Carcinoma (BCC) and Squamous Cell Carcinoma (SCC)) are diagnosed annually in the United States [11, 12]. In the

United States, these cancers affect more people than all other forms of cancer combined, posing a significant threat to public health [13]. Therefore, a full knowledge of the processes behind skin cancer is necessary for the development of adequate preventative and treatment methods and the optimal allocation of medical resources. Currently, there is a major emphasis on a study on the involvement of the immune system in the pathogenesis and development of skin cancer in order to develop innovative therapeutic strategies [12].

2.1. Classification of Skin Cancer

The most common cancer is skin cancer, which encompasses both non-melanoma and melanoma [14]. Non-melanoma skin cancer accounts for roughly 30% of all malignancies, and its prevalence is rising significantly [15]. BCC is the most widespread kind of skin cancer (75-80% of cases) and also the most common type of cancer in people [16]. SCC is the second most common form of skin cancer in the United States, behind BCC, with around one million cases each year. This kind of cancer might grow on healthy tissue or preexisting problems like actinic keratosis or an old burn scar. SCC risk factors include exposure to ultraviolet (UV) radiation, light skin, age, male gender, persistent lesions, radiation treatment, the presence of oncogenic viruses (*i.e.*, human papillomavirus (HPV)), environmental exposures, and immunosuppression [15, 17, 18]. Immunosuppressive therapy, whether physiological or pharmacological, is related to a higher cancer risk relative to the general population, suggesting the importance of immune system disorders in the genesis and development of skin cancer [19].

Melanoma represents the most lethal form of skin cancer and places a substantial load on the healthcare system. Melanoma, unlike other malignancies, affects youthful, more socially active individuals with a median age of 57 at diagnosis. Hereditary and environmental factors contribute to the development of skin cancers. Despite ongoing research to create innovative therapeutic modalities, the prospects for those with severe illnesses remain dismal [20]. In the general population, cutaneous lymphoma, Kaposi sarcoma, Merkel cell carcinoma (MCC), skin adnexal tumors, and skin sarcoma are the most prevalent forms of skin cancer [21]. MCC, a neuroendocrine epidermal tumor, is a unique and aggressive form of skin cancer. Merkel cell polyomavirus (MCPyV) is responsible for the majority of cases, however other risk factors including advanced age, UV exposure, and immunosuppressive therapy have also been documented [22].

CHAPTER 4

Probiotics-based Anticancer Immunity In Colon Cancer

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Abstract: Probiotics are live microorganisms, which confer a health benefit to the host after administering them in adequate amounts. Health benefits of probiotics include antimicrobial activity and gastrointestinal infections, effectiveness against diarrhoea and *Helicobacter pylori* infection, improvement in lactose metabolism, reduction in serum cholesterol, inflammatory bowel disease, immune system stimulation, anti-mutagenic properties, and anti-carcinogenic properties. Since probiotics exhibit a positive health impact, many researchers pay attention to the role of probiotics in the enhancement of the immunological response of the host and also in colon cancer prevention and treatment. Probiotic strains, either live or dead cells, belong to the genera *Lactobacillus* and *Bifidobacterium,* which are typically evaluated for their immunomodulatory effect on the immune system. These strains can improve the immunological response both *in vitro* and *in vivo*. Many mechanisms of probiotics in the prevention and treatment of colon cancer have been proposed. Several studies demonstrate that probiotics and synbiotics exert an anti-carcinogenic effect on colon cancer cells (*in vitro)* as well as in clinical trials *(in vivo)*. These studies illustrate that probiotics and synbiotics are applied as adjunctive or alternative therapeutic agents for colon cancer management.

Keywords: Colon cancer, Immunomodulatory effect, Lactic acid bacteria, *Lactobacillus*, *Bifidobacterium*, Probiotics, Preventive effect.

1. INTRODUCTION

One hundred trillion microorganisms residing in the gastrointestinal tract (GIT), which are known as gut microbiota, provide essential health benefits to their host, especially by regulating immune homeostasis [1]. Non-specific and specific immune system components' development is stimulated by the GIT microbiota

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Mitesh Kumar Dwivedi, Alwarappan Sankaranarayanan & Sanjay Tiwari (Eds.)

just after birth and during the entire life and it functions as an anti-infectious barrier by suppressing the adherence of pathogenic microorganisms and subsequent cellular substratum colonization and by the production of bacteriocins and other toxic metabolites [2]. Gut microbiota dysbiosis has been associated with many disorders, including inflammatory bowel disease (IBD), obesity, asthma, psychiatric illnesses, and cancers [3]. Probiotics are defined as live microorganisms, which, when administered in adequate amounts, confer a health benefit to the host [4]. Health benefits of probiotics include antimicrobial activity and gastrointestinal infections, effectiveness against diarrhoea, improvement in lactose metabolism, reduction in serum cholesterol, immune system stimulation, anti-mutagenic properties and anti-carcinogenic properties. *Helicobacter pylori* bacterium has been recognized as an important cause of chronic gastritis and peptic ulcer and is a risk factor for gastric carcinoma [5]. Therefore, many investigations have focused on probiotics that exert the anti-*H. pylori* activity. *Lb. fermentum* P43-01 produced bacteriocin-like compounds which showed a broad spectrum of antimicrobial activities against *H. pylori* strains isolated from patients [6]. Due to the several advantages of probiotics to host's health, researchers have paid attention to them. Many researchers report about the immunomodulatory activity of probiotics on the immune system. These useful microorganisms in both live and inactivated forms can enhance the immune responses in colon cancer cells [16 - 22]. In addition, the application of probiotics or synbiotics in mice models resulted in the improvement of anti-inflammatory activity and reduction of proinflammatory cytokines levels [20, 23 - 28]. Numerous research works showed that probiotics and synbiotics exert preventive effects against cancer carcinogenesis in *in vitro* and *in vivo* models [17, 28, 30 - 41]. Moreover, several clinical studies have illustrated that probiotics or synbiotics can be used as alternative or adjunctive therapeutic substances in patients with colon cancer [42 - 49]. This chapter summarizes the updated knowledge of the effect of probiotics on the modulation of immune responses in both *in vitro* and *in vivo* models. The mechanisms of probiotics related to the prevention and treatment of colon cancer are also described. Furthermore, the scientific evidences associated with the preventive and treatment effects of probiotics/synbiotics in cell lines, animal models and clinical trials are also reviewed.

2. PROBIOTICS AND THEIR SELECTION

A United Nations and World Health Organization Expert Panel defines the term "probiotics" as *live microorganisms, which, when administered in adequate amounts, confer a health benefit to the host* [4]. Microorganisms typically used as probiotics belong to the heterogeneous genera of lactic acid bacteria (LAB) [*Lactobacillus* (*Lb.*), *Enterococcus* (*E.*), *Streptococcus* (*S.*), *Lactococcus* (*Lc.*),

Pediococcus (*P.*), and *Leuconostoc* (*Ln.*), and *Bifidobacterium* (*B.*)]. Moreover, some strains of *Bacillus*, *Escherichia* and yeast are also included as probiotic microorganisms (Table **1**) [7 - 9].

Table. (1). List of microorganisms used as probiotics [8, 9].

***Lactobacillus* (*Lb.*)**	***Bifidobacterium* (*B.*)**	**Other LAB**	**Other microorganisms**
Lb. fermentum	*B. longum*	*S. thermophilus*	*Bacillus subtilis*
Lb. reuteri	*B. bifidum*	*S. diacetylactis*	*Bacillus clausii*
Lb. mesenteroides	*B. animali*	*S. intermedius*	*Escherichia coli* strain Nissle
Lb. plantarum	*B. adolescentis*	*P. acidilactici*	*Saccharomyces cerevisiae*
Lb. rhamnosus	*B. breve*	*E. faecium*	*Saccharomyces bourlardii*
Lb. casei	*B. infantis*	*Lc. lactis*	-
Lb. paracasei	*B. lactis*	*Ln. mesenteroides*	-
Lb. johnsonii	*B. thermophilum*	-	-
Lb. acidophilus	-	-	-
Lb. lactis	-	-	-
Lb. crispatus	-	-	-
Lb. delbrueckii	-	-	-
Lb. farciminis	-	-	-
Lb. gasseri	-	-	-

The selection of probiotic strains is based on safety, functional properties and technological compatibility. Microorganisms that are used as probiotics should meet the terms of GRAS (Generally Recognized as Safe) in the United States and QPS (Qualified Presumption of Safety) status, considering the European Food Safety Authority (EFSA). The safety aspect of probiotic cultures includes the absence of pathogenicity and antibiotic resistance [10]. Probiotic strains must be identified to the strain level by phenotypic and genotypic characterization and then their safety assessment from historical evidence or experimental trial is carried out. LAB are known as GRAS and many *Lactobacillus* and *Bifidobacterium* species are intestinal gut microbiota. However, the safety assessment is still required because some LAB strains are associated with opportunistic infections [11]. Functional or probiotic properties are crucial criteria for which the probiotic strains must be evaluated. Microorganisms which are classified as probiotics must have basic properties such as resistance to gastric juices, exposure to bile, proliferation and colonization at the digestive tract.

CHAPTER 5

Probiotics Based Anticancer Immunity in Colorectal Cancer

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Abstract: Colorectal cancer (CRC) is the third most common cancer, originating in the colon and rectal region, leading to abnormal growth in the colon or rectal region. The gut microbiota plays a critical role in the maintenance of gut homeostasis, and dysbiosis in the gut microbiota has been associated with CRC pathogenesis. Probiotics can manipulate the gut microbiota, which can be effective in CRC treatment. Additionally, probiotics, through the modulation of host immune response, inhibition of tumor growth, reduction of microbial infection, inhibition of cancerogenic compounds, and regulation of apoptosis, can become a novel therapeutic option for the prevention and treatment of CRC. Therefore, this chapter mainly focuses on the mechanisms of probiotics-based anticancer immunity in CRC, so the existing knowledge could help in developing a safe and effective treatment for CRC.

Keywords: Apoptosis, *B. bifidum*, Colorectal cancer (CRC), Dysbiosis, Gut microbiota, *L. rhamnosus*, *L. acidophilus*, Probiotics, Short chain fatty acids (SCFAs), Tyrosine kinase.

1. INTRODUCTION

Colorectal cancer (CRC) originates in the colon or rectal region, leading to abnormal growth [1]. CRC emerges in the large intestine, where the heightened replication of the epithelial cells gives rise to benign adenoma, which can metastasize and result in carcinoma [1]. CRC is preventable, if there is early detection of growth or polyps in the colon, which can develop into cancer cells [2]. Symptoms include abdominal pain, weight loss, weakness, fatigue, diarrhoea, constipation, blood in stools, bleeding in rectum, changes in consistency of stool, *etc* [3]. The risk factors for CRC include family history, age, diabetes, smoking, alcohol, inflammatory bowel disease, lifestyle, *etc* [4]. It is one of the most

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Mitesh Kumar Dwivedi, Alwarappan Sankaranarayanan & Sanjay Tiwari (Eds.)

diagnosed cancers worldwide, and its prevalence is rising in developing countries [3]. In 2020, there were approximately 1.9 million new cases of CRC, which are estimated to be responsible for 93,500 cancer deaths [3]. A higher incidence of CRC is found in males compared to females, and approximately 4.4% of males and 4.1% of females are estimated to be affected by CRC in their lifetime [5].

Currently, surgeries and chemotherapy are the first choice of treatment for CRC. Chemotherapy includes single agent and multiple agent regimens, but limitations include systemic toxicity, unsatisfying response rate, and low specificity. Additionally, surgeries are difficult for patients in advanced stages of CRC [6]. Moreover, for patients with unresectable lesions or who are intolerant to surgery radiotherapy and chemotherapy, treatments which can lead to maximum shrinkage of tumor are the only options [6]. Therefore, there is a need to develop novel approaches for treatment of CRC.

Probiotics are live microorganisms residing in the gastrointestinal tract (GIT) that confer health benefits to the host [7]. Probiotics organisms have specific characteristics, including gastric acid, bile salt stability and the ability to adhere and colonize the intestinal mucosa [7]. Additionally, probiotics provide essential nutrients to the host and help in metabolizing indigestible compounds in the host gut [8]. Additionally, probiotics provide immunity and maintain homeostasis *via* production of short chain fatty acids (SCFAs) such as acetate butyrate, *etc* [8].

In the case of CRC, the intestine is characterized by high proportion of bacteria that cause inflammation in the GI tract [9]. Probiotics can reduce the bacteria from the intestine that contribute to CRC [10]. Moreover, probiotics through the modulation of host immune response, inhibition of tumor growth, reduction of microbial infection, inhibition of cancerogenic compounds, and regulation of apoptosis, can serve a novel therapeutic option for CRC [10]. Multiple strategies including probiotics, prebiotics, synbiotics and fecal microbiota transplantation (FMT) can be used to treat CRC [11]. Therefore, this chapter discusses the mechanisms of probiotics based anticancer immunity in CRC, provides animal and human clinical trials evidences for these probiotics use in CRC treatment and prevention, in addition to the role of gut microbiota in CRC development.

2. PATHOGENESIS OF COLORECTAL CANCER

The pathogenesis of CRC is complex; multiple factors, including smoking, diabetes, inflammatory bowel disease, eating habits, obesity, alcohol consumption, and genetics are involved in the progression of CRC [4]. CRC can be sporadic, inherited, or arise from inflammatory bowel disease (IBD) [12].

Chromosome instability pathway, microsatellite instability pathway, and CpG Island Methylator Phenotype (CIMP) pathway are mainly responsible for sporadic CRC cases [12]. The chromosomal instability arises from either gain or loss of chromosomes, which leads to activation of oncogenes such as KRAS and BRAF, inhibiting tumor suppressor genes and resulting in CRC tumorigenesis [12, 13]. Microsatellite instability (MSI) pathway is another mechanism of sporadic CRC development [12]. It refers to the biochemical detection of frame shifted microsatellite sequences from genomic DNA. This MSI leads to frameshift mutations in the coding region of tumor suppressor genes or oncogenes contributing to carcinogenesis [12]. For the CIMP pathway, epigenetic instability in CRC has been demonstrated, and global hypomethylation was associated with genetic instability and chromosomal aberrations [12]. Moreover, hypermethylation of the promoter region of tumor suppressor genes can lead to cancer initiation. Additionally, the altered methylation patterns affect cell cycle regulation, transcription, cell-cell adhesion, invasion, and metastasis [14].

The hereditary CRC is most inherited in autosomal dominant pattern; however *MUTYH*-associated polyposis and NTHL1 are also inherited in autosomal recessive patterns [15]. Evidence of hereditary CRC comes from the family history of CRC, presence of multiple other cancers in patients, and early age diagnosis of CRC [15]. Hereditary CRC is mainly of two types: i) polyposis, which includes familial adenomatous polyposis (FAP) and attenuated FAP (genetic variation in *APC* gene) and *MUTYH* associated polyposis (genetic variation in *MUTYH* gene) [16], and ii) lynch syndrome caused by genetic variation in the *MMR* genes (*MLH1, MSH2, MSH6,* and *PMS2)* and *EPCAM* genes [17].

Patients with IBD have a 60% higher risk of developing CRC. Chronic mucosal inflammation is the primary cause of CRC carcinogenesis in IBD patients [18]. The factors that are responsible for increased risk of CRC in IBD patients are active inflammation, primary sclerosing cholangitis, family history of CRC, extent and duration of severe inflammatory conditions, cytokine secretion, and shortened tubular colon and vascularization, significantly increased the risk of CRC in IBD patients [18]. However, folic acid use, ursodeoxycholic acid, 5-ASA treatment, colectomy, and compliance with CRC surveillance guidelines can prevent CRC development in IBD patients [6].

3. CORRELATION BETWEEN THE GUT MICROBIOTA AND COLORECTAL CANCER

Dysbiosis in the gut microbiota and infections with bacteria can lead to the occurrence of CRC [19]. The dysbiosis of gut microbiota, infections, and

CHAPTER 6

Probiotics-based Anticancer Immunity in Breast Cancer

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Abstract: A growing number of evidence is available in support of the advantageous role of a balanced intestinal microbiota in the progression and manifestation of malignant tumors, not only in the gastrointestinal tract but in other distant tissues as well, with the most potential role in breast carcinoma. Breast cancer involves a complex interplay of several factors, such as familial history, use of hormonal replacement therapy, dietary habits, lifestyle, environment, clinical features, genetics and epigenetics. Recently, a positive correlation between a patient's breast microbiome and cancer has beocme a novel potential risk factor. In the present chapter, we tried to discuss the role of microbiome as a potential breast cancer risk factor and tried to investigate the literature focussing on the proposed mechanisms behind the interaction of microbiome, human genetic makeup involved in the onset of breast carcinogenesis and determining the effect of transformed breast, milk and gut microbiome on the physiological status of both normal and malignant breast. We also tried to shed light on the resistance to chemotherapeutic treatment among individuals with altered microbiomes with an emphasis on the role of the microbiome in developing and maintaining inflammation, epigenetic alterations and estrogen metabolism. Interestingly, bacterial species are indispensable modulatory agents of widely used chemotherapeutic/ immunotherapeutic regiments. But the exact role of commensal bacteria in immunity, formation of neoplasia and response to treatment needs much more research because most of the available knowledge is based on animal model studies and needs its translation to humans which requires great precision and has various hurdles too. Therefore, we tried to give a comprehensive overview of current knowledge in terms of breast cancer therapeutics and suggest integrating probiotic bacteria and/or modulation of the intestinal microbiota to be used as immune adjuvants, targeting to enhance the effectiveness of conventional anti-tumor treatments and cancer immunotherapies as well.

Keywords: Breast microbiome, Breast cancer, Cancer therapeutics, Immunotherapies, Probiotic bacteria.

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Mitesh Kumar Dwivedi, Alwarappan Sankaranarayanan & Sanjay Tiwari (Eds.)

1. INTRODUCTION

Breast cancer is the 2nd most commonly occurring cancer and is the main cause of cancer-related deaths worldwide [1]. There are several factors that influence the initiation and aggressiveness of tumor, including genetic and environmental. A comparatively new agent responsible for cancer is probiotics (the beneficial microorganisms, *e.g.*, bacteria). Once cancer appears, the body responds by activating the immune cells of the body. Immune cells and epithelial cells of mucosal surface bidirectionally interact with the bacterial population present in the gastro-intestinal tract (GIT) of humans [2]. The bacterial population in GIT are dynamic, and they can perform multiple functions of metabolizing bile salts, synthesizing vitamin B and K, and regulating cytokine production for fighting against pathogenic organisms. The population of probiotics is easily altered by numerous factors like pharmaceutical use, infections, travelling to international destinations, diet, race, ethnicity and age. Changes in the GIT bacterial population is an ongoing process, therefore this means that they tend to change throughout the lifetime of an individual [3]. Various diseases like asthma, arthritis, diabetes and obesity are also linked with a change in the bacterial flora. It has been found in many studies that the excessive use of antibiotics leads to tumor development which means these bacteria have a role in carcinogenesis as well [4].

Keeping in mind the overall importance of bacteria for human health, the term probiotic was first coined by Elie Metchnikoff 100 years back. Later probiotics were defined by WHO (World Health Organization) and FAO (Food and Agricultural Organization) as 'live microorganisms which, when administered in adequate amounts (in food or as a dietary supplement) confer a health benefit on the host'. Some of the most commonly used strains of bacteria for the purpose of probiotics are summarised in Fig. (**1**). A number of probiotics stay in the GIT, and they vary from person to person and are known to reduce carcinogen exposure by restricting their absorption [5]. In the case of colon cancer, they are known to alter physio-chemical conditions, produce anti-mutagenic components, degrade carcinogens, alter the activity of metabolic micro-flora and enhance the immune response of the host [6]. In this chapter, detailed information is provided regarding how these probiotics affect breast tissue and their effects on the treatment of breast cancer patients.

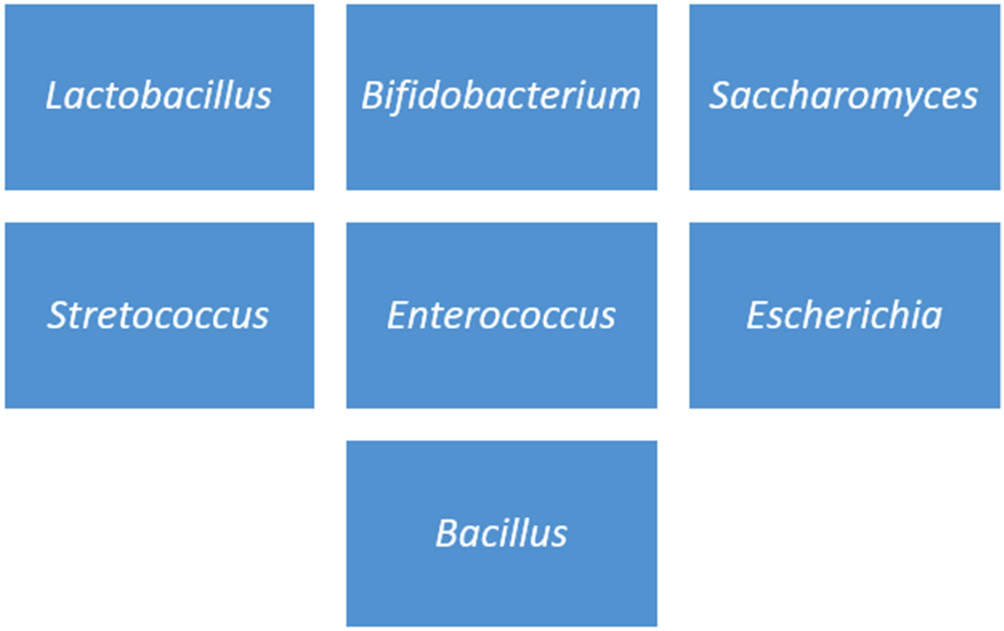

Fig. (1). Most commonly used bacteria as probiotics.

Most cancer therapies are immune-based, but unluckily, breast cancer does not respond to these immune checkpoints. Breast cancer patients are mostly kept on antibiotics that destroy the microbiota irrespective of harmful or beneficial bacteria and give rise to resistant strains that kill people all over the world; additionally, not all breast cancer patients respond well to antibiotics [7]. In order to overcome these problems, it is important to understand what the normal microbiota looks like and which bacteria are the probiotics, and which drugs can be administered to these patients (personalized treatment). Another observation is that whatever applies to breast cancer may not be applicable to all cancers and *vice versa* [8].

2. GUT MICROBIOME

Numerous viruses and microorganisms are present in the human body that usually exceed the number of human cells [9]. These microorganisms are collectively known as microbiota. It consists of different communities of archaea, viruses, eukaryotes, and bacteria and affects our health by cross-talking with one another [10]. Ideally, this should be an organ of our body that is forgotten and is mostly present in the GIT. It is estimated that most (35%) of cancers are due to diet, and one of the most prominent among them is breast cancer (50%) [11]. If the diet is a

CHAPTER 7

Probiotics Based Anticancer Immunity In Stomach Cancer

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Abstract: Stomach cancer is a global health challenge due to its increasing prevalence. The intestinal microbiota of humans plays a vital role in producing short-chain fatty acids, developing resistance towards pathogenic microbes, nutrient absorption, modulation in immunological response, metabolism, synthesis of vitamins, and gut immune system development. Many diseases or disorders, including cancers, obesity, psychiatric illnesses, rheumatoid arthritis, and inflammatory bowel syndrome, are associated with an imbalance of microbiotas. Earlier reports suggest that probiotics *via* the oral route act as a functional food and suppress cancer development. Further, some probiotics are clinically effective in reducing post-operative inflammation in cancer patients. Probiotics primarily display inhibitory effects against *H. pylori* infections in the digestive tract. The combination of probiotics with antibiotics has effectively eradicated *H. pylori* infections. Besides, probiotics reduce the pro-carcinogens metabolism, they also diminish the growth of pathogens and improve the consistency of the intestinal barrier. Moreover, compounds produced by the microorganisms are reported to interact unswervingly with cancer cells and affect their survival. The therapeutic efficacy and adverse side-effects of the strategies used for stomach cancer prevention could be improved by using probiotics either as adjuvant or neo-adjuvant as the safety concern of the commercially used strains has been verified. The underlying mechanism describing microbiota's effect on oncogenic activation, carcinogenic metabolite production, DNA damage, inhibition of tumour immunity, and chronic inflammation induction still needs a more detailed investigation. In addition, double-blind, placebo-controlled, randomized, and well-designed clinical studies are required to understand the efficacy and mode of action to reduce the death rate and stomach cancer burden. In depth studies are essential to set probiotics as an eccentric strategy for stomach cancer prevention and treatment.

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Mitesh Kumar Dwivedi, Alwarappan Sankaranarayanan & Sanjay Tiwari (Eds.)

Keywords: Gut immune system, *H. pylori*, Immune system, Inflammation, Intestinal microbiota, Probiotics, Stomach cancer, Tumour immunity.

1. INTRODUCTION

Inflammation influences several biological processes involved in the progression and development of a tumour and acts as an intrinsic tumour characteristic. Non-immune cells, neoplastic and immune cells coexist in tumours, but tumour growth is compromised when an appropriate microenvironment is unavailable [1, 2]. The heterotypic signalling in non-neoplastic and neoplastic cells reshaped the tumour niche. The emergence currently saw a change in the treatment paradigm of the therapies centered on tumour microenvironment [3, 4]. Among the most widely used immunotherapeutics, immune-checkpoint blockers (ICBs) are the principal approaches in cancer [5 - 7]. Despite some significant results in a few cancer types, only a particular type of population gets the beneficial effects. The major challenge is identifying the accurate and precise biomarkers in the clinical setup and personalising an immune treatment [8].

In stomach cancer, chronic inflammation and infection are essential for stomach cancer pathogenesis. The *H. pylori* infection is linked to the intestinal and stomach cancers that trigger persistent and chronic inflammation in gastric mucosa, inflammatory cell infiltration, and diverse inflammatory mediators [9]. Epstein-Barr virus (EBV) is also linked to 10% of total stomach cancer, and $CD8^+$ T cells infiltration is the characteristic feature of EBV-positive stomach cancer [10]. Genetic and environmental determinants play an essential role in the genesis of stomach malignancy; thus, at the molecular level, the ecological and genetic factors make it a complex and heterogeneous disease that increases the clinical complexity. FDA approves the use of pembrolizumab, a programmed cell death protein 1 inhibitor for the recurrent and advanced PD-L1 expressing stomach cancer [11]. Earlier studies indicate that the alliance of PD-1 and PDL-1 expression correlates with the clinical parameters and the survival of patients with stomach cancer [12 - 15]. Besides, few studies suggest inducing PD-L1 expression by *H. pylori* [16 - 19].

Moreover, several other parameters have now been accepted as biomarkers due to the significant clinical relevance for predicting the response against ICBs studied in stomach cancer. Hence this book chapter summarises epidemiology, histology and immune contexture concerning stomach cancer. Further, we discuss the significance of predictive markers concerning the immune microenvironment. Moreover, we include the probiotics, their impact on stomach cancer management, and the probiotics' clinical trials. In addition, we shed light on the

significant current gaps in the treatment, diagnosis, prognosis, and prevention of stomach cancer and the challenges that need to be addressed.

2. EPIDEMIOLOGY OF STOMACH CANCER DEVELOPMENT, SYMPTOMS AND RISK FACTORS

Stomach cancer is developed from the stomach lining and is also known as gastric cancer. It is the fifth-leading cancer among the other cancer types, with 7% cases and a 9% death rate; it is the third most common cancer type with the leading cause of death [20]. The stomach lining comprises glands and columnar epithelial cells sensitive to inflammation, especially gastritis, leading to gastric ulcers and gastric cancer [21]. According to the American Institute of Cancer Research (2022), approximately 26,380 new cases and 11,090 deaths were recorded in the United States. It is the fourth most common cancer type among men, while it is the seventh most common cancer type in females in India [22]. According to the statistical analysis, South Korea showed the highest stomach cancer rates, followed by Mongolia in 2018. The order among countries from the highest to the lowest stomach cancer incidence rates was as follows: South Korea > Mongolia > Japan > China > Bhutan > Kyrgyzstan > Chile > Belarus > Peru > Vietnam.

2.1. Incidence and Mortality

Stomach cancer is a more prevalent cancer type in males than females, with 2.2 times in developed countries while the ratio is 1.83 in developing countries. It is the most frequently diagnosed type of cancer [23]. The highest incidences were recorded in Latin America and Central and Eastern Asia. The highest incidence of 60/100000 has been reported in the Republic of Korea [24]. Approximately 783,000 deaths were recorded each year, making it the third most deadly type of cancer in males. It is attributed to about 8.3% of total cancer deaths, and the cumulative death risk is 0.57% in females and 1.36% in males from birth to the age of 74 years [20].

2.2. Geographical Variability and Trends

In the United States, the 5-year survival rate is 31%, indicating that most of the cases were diagnosed at the metastatic stage. Approx 67% of the survival rates were observed for pre-metastatic stomach cancer diagnosis. In Asia, the 5-years survival rate is 12% higher due to early diagnosis by lymph nodes examination, while 19% and 15% survival rate is reported in the United Kingdom for 5-years and 10-years survival, respectively. Europe shows a 26% survival rate, higher than the United Kingdom but less than the United States [25 - 27].

CHAPTER 8

Probiotic-based Anticancer Immunity In Hepatocellular Carcinoma (liver Cancer)

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Abstract: One of the most dreaded outcomes of chronic liver illness is hepatocellular carcinoma (HCC), and it is the most prevalent primary liver cancer. The gut-liver axis has been shown to play a key role in the emergence of chronic liver disorders, including HCC, in recent experimental and clinical studies. The altered gut microbiota is becoming well recognised as an important factor in the progression of chronic liver disorders, such as HCC. Probiotics administration has been proposed as a new, safe and cost-effective strategy for preventing or treating HCC. Probiotics' ability to bind carcinogens, regulation of gut microbiota, improvement of intestinal barrier integrity, and immunomodulation are the mechanisms by which they exert anticancer benefits. This chapter discusses the alterations in gut microbiota linked to HCC and the implications of probiotics and prebiotics for anticancer mechanisms towards HCC.

Keywords: Carcinogens, Gut microbiota, Hepatocellular cancer, Liver cancer, Probiotics, Prebiotics, Short chain fatty acids (SCFAs).

1. INTRODUCTION

Hepatocellular carcinoma (HCC) is primary liver cancer among the most fatal health conditions which are rapidly expanding across the globe [1, 2]. It has been predicted that by the year 2025, more than 1 million individuals will be annually affected by hepatocellular carcinoma [3]. HCC accounts for approximately 90% of cases and among this, 50% of cases results due to Hepatitis B virus (HBV) infection, which is an important risk factor for HCC [4]. HCC is majorly caused due to chronic liver disease, non-alcoholic fatty liver disease (NAFLD), alcohol abuse, diabetes, *etc* [5 - 7]. HCC can be treated well upon early diagnosis of HCC in patients [8]. But unfortunately, the diagnosis of HCC is often made in later disease stages, which is mostly accompanied by liver failure [9].

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Mitesh Kumar Dwivedi, Alwarappan Sankaranarayanan & Sanjay Tiwari (Eds.)

The human gut consists of 100 trillion microorganisms of diverse taxonomy collectively known as gut microbiota, but the human gut contains more genes than the human genome [10]. Immediately after birth, commensal bacteria colonize within the host and support the overall maintenance of health. Intestinal microbiota helps in degrading bile acids, aids in digestion, produces vitamins, helps in modulating immunity, and also helps in treating diseases such as cancer [11 - 13]. In a healthy individual, *Firmicutes*, *Bacteroidetes* and *Actinobacteria* account for the majority of the bacterial phyla [12]. Studies have revealed that gut microbiota and gut microbiota-derived products play an indispensable role in the pathogenesis and treatment of HCC. For example, lipoteichoic acid and deoxycholic acid induced the expression of prostaglandin-endoperoxide synthase 2 or cyclooxygenase-2 (COX-2) in senescent hepatic stellate cells (HSCs) *via* Toll-like receptor 2 (TLR-2) to amplify prostaglandin E2 (PGE2)-mediated inhibition of antitumor immunity, resulting in HCC [14]. It has been observed that gut microbiota-derived products can influence non-alcoholic steatohepatitis (NASH) and virus-induced HCC progression *via* modulating hepatic inflammation and immunology [15]. In comparison to non-responders, HCC patients who responded to anti-programmed cell death protein 1 (anti-PD-1) treatment showed a higher taxonomic richness in faeces [16]. Maintaining a balanced microbiota composition is critical. Intestinal dysbiosis occurs when this micro-ecology is disrupted, resulting in an overgrowth of particular harmful bacteria that can cause a number of disorders, including liver pathology. Indeed, the gut microbiota composition is altered in many liver disorders, making gut microbiota remodelling a promising therapeutic target. Probiotics, as a functional food ingredient, may have a positive impact on the gut microbiota and alter the aetiology of chronic liver illnesses [17], and new research suggests that probiotics could be utilised as a treatment for HCC [18] (Table **1**). The interaction of gut microbiota with HCC and the potential therapeutic implications of probiotics for HCC are discussed in this chapter.

Table 1. Clinical studies of probiotics and prebiotics in liver cancer (HCC).

Title of the Study	Status	Condition	Interventions	Clinical Trials.gov Identifier
Probiotics enhance the treatment of PD-1 inhibitors in patients with liver cancer	Recruiting	Liver cancer	Experimental: probiotics group The oral probiotic (*Lactobacillus rhamnosus* Probio-M9, one times a day during the whole treatment)	NCT05032014

(Table 1) cont.....

Title of the Study	Status	Condition	Interventions	Clinical Trials.gov Identifier
Integrative nutrition care plan for the patient with liver and colorectal cancer	Enrolling	Liver and colorectal cancer	Powdered supplement 1 (containing β-glucan and gamma-aminobutyric acid) and nutritional products and powdered supplement 2 (contains water-soluble dietary fibre and probiotics)	NCT05030090
Influence of probiotics administration before liver resection in liver disease	Completed	Liver fibrosis, liver cirrhosis and hepatocellular carcinoma	Probiotics- Lactibiane Tolerance Active substance mixture of lactic 10% *Bifidobacterium lactis* LA 303, 10% *Lactobacillus acidophilus* LA 201, LA 40% *Lactobacillus plantarum* 301, 20% *Lactobacillus salivarius* LA 302, LA 20% *Bifidobacterium lactis* 304 Dosage: 10 X 10^9 probiotic / capsule Composition: One capsule of 560 mg contains Lactibiane tolerance: • 345 mg of corn starch • 114 mg premix lactic • 6 mg of magnesium stearate	NCT02021253
Probiotics in the prevention of hepatocellular carcinoma in cirrhosis	Not yet recruiting	Hepatocellular carcinoma	Each 50 ml bottle contains *Lactobacillus casei* 3.3 x 10^7 CFU / day, *Lactobacillus plantarum* 3.3 x 10^7 CFU / day, *Streptococcus faecalis* 3.3 x 10^7 CFU / day and *Bifidobacterium brevis* 1.0 x 10^6 CFU / day (BIOFLORA®, BIOSIDUS SA, Argentina)	NCT03853928
Prebiotic effect of eicosapentaenoic acid treatment for colorectal cancer liver metastases	Recruiting	Liver cancer	Drug: Icosapent Ethyl Oral Capsule Soft gelatin capsules containing 1g pure EPA-EE	NCT04682665

2. ROLE OF GUT MICROBIOTA IN LIVER DISEASES

For nutrient absorption and metabolism, the stomach and liver are critical organs. According to new findings, there is a strong link between the liver and the intestines. The hepatic portal vein supplies the liver with roughly 75% of its blood supply. The intestinal blood transports nutrients from the gut to the liver, where

CHAPTER 9

Probiotics-based Anticancer Immunity In Cervical Cancer

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Abstract: In the recent past, many investigations have been directed toward finding the possible relationship between probiotic preventive-therapeutic effects and different cancers. Among different cancers, human papillomavirus (HPV)-induced cancer is the third most frequent cancer among women, resulting in being the second cause of death worldwide. Current treatments, such as chemotherapy and radiotherapy, have been shown to have some limitations, and the available effective cervical vaccines are costly, particularly in developing countries. Therefore, the researchers seek alternatives, such as natural components, as a new approach to treating and cure HPV-induced cancer. Among several natural components, probiotics have increasingly gained more attention due to the probiotic-associated immunomodulation and therapeutic efficacy shown in several studies, as well as their lower risk for human health. In this chapter, we have reviewed the association between probiotics and cervical cancer and discussed how probiotics could exert their effects to suppress or even inhibit the growth of cervical tumors, preclinically or clinically. The different aspects of probiotic application have been precisely studied to assess the potential of probiotics in improving or treating HPV-induced cancer. In addition, the effects of probiotics on immune responses have been described.

Keywords: Anti-cancer effect, Cervical cancer, Human papillomavirus, Immunomodulatory effect, Immune response, Probiotics.

1. INTRODUCTION

A survey in 2018 demonstrated that about 311,000 women out of 570,000 women diagnosed with cervical cancer died from this disease [1, 2]. Notably, the incidence and mortality of cervical cancer are believed to be positively related to

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Mitesh Kumar Dwivedi, Alwarappan Sankaranarayanan & Sanjay Tiwari (Eds.)

human papillomavirus (HPV) and human immunodeficiency virus (HIV) infection, whereas being negatively associated with cervical cancer screening coverage [3]. During the 1990s, HPV was recognized as a key cause of cervical cancer, and this association is equally important as the discovery of the relation between cigarette smoking and lung cancer, or between chronic infections with hepatitis B virus (HBV) [4]. According to reports, genital HPV infection is responsible for approximately 500,000 cervical cancer cases and 275,000 associated deaths each year globally [5, 6]. Moreover, HPV has been attributed to playing a role, in varying degrees, in different diseases ranging from benign papillomas or warts to distinct cancers, including cancer of the anus, vulva, vagina, penis, and head and neck [7 - 10].

Papillomavirus, which is a member of the *Papovaviridae* family, is a relatively small, non-enveloped virus with a double-stranded DNA approximately 8 kilobases in length, and its genome is enclosed by a spherical capsid with icosahedral harmony and a diameter of about 55 nm. The genomic particle of this virus is hitched to cellular histones and contained in a protein capsid composed of 72 pentameric capsomers [11]. Three segments, including early, late and genomic regions, compose the genome of papillomavirus. E1, E2 and E4-E8, which are considered early regions, constitute half of the HPV genome. The E1 and E2 are involved in the regulation of DNA replication, E2 in transcription, and E5, E6 and E7 in cell transformation. The late region (L), including L1 and L2 capsid proteins, comprises 40% of the genome, while the genomic regulatory region, involving the structural proteins of the virion, forms the rest of the genome [12, 13].

Although there are more than 100 types of HPV, at least 14 types are known to cause cancer (also known as the high-risk type). In comparison to low-risk HPV 6 and 11, which cause genital warts and respiratory papillomatosis, long time infections with high-risk HPV types, including 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70, particularly 16 and 18, which together contribute to more than 70% of cervical cancers worldwide, result in cervical cancer [11, 14]. HPV primarily transmits through skin-to-skin contact, and studies have revealed that sexual activity influences the risk of being infected with HPV infection and cervical cancer [15]. There are four stages of cervical cancer occurrence; first, HPV infection of the transformation zone (TZ); second, HPV infection persistence; third, clonal expansion of HPV-infected cells to high-grade cervical intraepithelial neoplasia (CIN 3) or adenocarcinoma *in situ* (AIS); and forth, their progression to invasive cancer [16].

2. PATHOGENESIS OF HPV AND CERVICAL CANCER

When the virus particle anchors to the epithelial basal layer through its receptor, heparan sulfate proteoglycans (HSPGs), and enters the dividing basal cells, infection with papillomaviruses begins [1]. Following the entry of the virus, the viral genome is maintained and amplified in the nuclei of infected basal epithelial cells. In order to have a persistent infection of basal cells, viral genomes approximately replicate once per cell cycle during the S phase, making HPV genomes persist in basal epithelial cells for years to decades [1, 17]. Although the basal cells become infected, these cells keep dividing and generating daughter cells, one of which remains in the basal layer to continue replication, which is required for the replication of the virus. The other daughter cell goes upward toward the suprabasal layer and differentiates there till it is finally shed from the epithelial surface [18, 19]. E5, E6, and E7 actions, which lead to induction and propagation of cell growth, result in constant cervical cells' growth and division, to stimulate the late viral genes expressed in the suprabasal layer, needed for capsid structure formation. HPV life cycle continues over time, and carcinogenic progression is not often the normal HPV life cycle, so the vast majority of infections are benign and transitory, which ultimately become undetectable in 12-24 months [20, 21]. Compared to genital HPV infections caused by low-risk (LR) non-oncogenic HPVs, which show higher rates of viral clearance (clearance of 90% of infections within 2 years) and disease regression, the high-risk (HR) types like HPV 16 and 18, belonging to the α genus, are associated with cancers of the cervix [22]. In other words, infection with HR HPV leads to lesions in the cervix, inclining to progress toward invasive cervical cancer over time [23]. Among oncogenic HPV types, HPV types 16 and 18 have been shown to be in high-grade cervical dysplastic lesions and the majority of invasive cervical cancers. Additionally, HR HPVs have some strategies to overcome the dependency on host cellular DNA synthesis machinery as compared to LR HPV with a normal life cycle. One strategy is to integrate their genome into a host chromosome, which normally occurs near common fragile sites of the human genome [24]. In this process, *E6* and *E7* genes tend to constantly express while other portions of the viral DNA are deleted or their expression is disturbed [25]. While E2 and E1, the two proteins playing an important role in the transcription and replication control, lose their activities, the deregulation of E6 and E7 oncoproteins begins [26]. Studies have demonstrated that the expression of high-risk HPV *E6* and *E7* genes in primary human keratinocytes facilitates their immortalization [27]. In addition, the E6 and E7 proteins are pivotal for the inhibition of tumor suppressor genes such as *p53* and *pRb* (retinoblastoma protein) [28]. E6-mediated inhibition of p53 allows several cellular changes to turn a cell oncogenic. One of the changes is to elicit uncontrolled cell proliferation by evading the cellular checkpoints. The degradation of p53 occurs when HPV E6, with the help of E6AP, binds to a motif,

CHAPTER 10

Probiotics-based Anticancer Immunity In Lung Cancer

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Abstract: Among various death-causing diseases, the morbidity and mortality related to cancer are the highest, with millions of new malignancies added to the tally every year and predicted to increase at a higher rate by 2030. Lung cancer is continued to be the leading cause of cancer death worldwide, with a share of 11.6% of all cancers. Since the start of the millennium, there has been a continuous effort to provide the benefits of probiotics in the management and treatment of cancer, particularly lung cancer. Probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer health benefits on the host". These include specific strains of bacteria and fungi. Bacterial strains belonging to *Lactobacillus* and *Bifidobacterium* have demonstrated promising results in the prevention, attenuation, and treatment of the progression of lung cancer. The present chapter focuses on the types and aetiology of lung cancer and the role and mechanism of action of probiotics in providing immunity against lung cancer.

Keywords: Chemotherapy, Dysbiosis, Dendritic cells, Immunity, Immuno-modulation, Immune checkpoint inhibitor ICI), Interleukins, Lung cancer, Microbiome, Mutagens, Natural killer T (NKT) cell, Probiotics.

1. INTRODUCTION

Lung cancer is categorized as the most common cancer (a share of 11.6% of all cancers), with the highest rate of mortality (1.7 million) out of over 2.09 million

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diagnoses in 2018 [1, 2]. The overall survival rate of five years in lung cancer is very low (≈ 20%) because of reasons such as delayed diagnosis and low response rate to the prescribed treatment [3]. The major factors contributing to lung cancer include genetic factors, smoking, drinking of alcohol, chronic respiratory diseases (such as asthma and chronic obstructive pulmonary disease, COPD), environmental factors (*e.g.*, microbial population in humans, microbiota, *etc.*), heavy metal consumption, exposure to silica dust, radon gas and asbestos [4, 5]. Apart from host microbiota, non-native pathogens also play an important role in the mediation of oncogenesis, including human papillomavirus (HPV), Kaposi's sarcoma virus (KSV), and Epstein-Barr virus (EBV) [6]. In addition, chronic antibiotic therapy can increase the risk of oncogenesis in the lung by killing the helpful microbes involved in the maintenance of homeostasis, providing resistance against disease-causing pathogens, and regulating the normal functioning of the system. Among all the factors, smoking is the major contributor accounting for 80-90% of all lung cancer cases [7].

Treatment options available for lung cancer are surgery, chemotherapy, radiotherapy, targeted therapy, and immunotherapy. But the treatment of lung cancer is based on the stage of cancer and unique health condition [8]. For instance, early-stage lung cancer needs surgery for remedy, whereas, for the advanced stage, the best treatment options have been chemotherapy, radiotherapy, immunotherapy, or a combination thereof [8, 9]. The current management of lung cancer involves systemic chemotherapy, which arbitrarily kills cancer cells and damages healthy cells, and may lead to drug resistance. Furthermore, it also gives birth to life-threatening side effects by compromising immunity and strength and impairing the treatment option, which mostly leads to suffering worse than the malignancy of cancer itself [10]. Despite the modern toll for diagnosis and various methods for treatments, the mortality related to lung cancer is still high. Therefore, to reduce mortality and minimize the suffering due to side effects caused by advanced treatment, scientists are more focused on alternative therapies, including physical activity, lifestyle modification, and nutrition supplements. The outcome of that effort is probiotics, which are currently used extensively in the management of various diseases, including cancers that originate in different body parts [11].

According to the United States-World Health Organization (US-WHO), probiotics are defined as live microorganisms which, when administered adequately, confer health benefits on the host [12, 13]. Probiotics are generally recognized as safe (GRAS) as they are frequently derived from safe microbes, such as *Lactobacillus* (naturally present in the small intestine) and *Bifidobacterium* (natural inhabitant of the large intestine) [14]. They are used to prevent and treat various cancers, including lung cancer, and act by enhancing innate immunity and antagonizing

the pathogenic organisms in airways. Thus, probiotics are believed to act as immunomodulatory agents, and host defense activators, and decrease the disease severity [15]. The first probiotics introduced into research were *Lactobacillus acidophilus* by Hull *et al*. in 1984 and then *Bifidobacterium bifidum* in 1991 by Holcombh *et al*. [16]. The present chapter discusses lung cancer, drivers for lung cancer, ideal characteristics, and mechanism of action of probiotics as immunotherapy in preventing and treating lung cancer.

2. LUNG CANCER

Cancer is an uncontrolled cell division caused by unrepairable damage of deoxyribonucleic acid (DNA), and originates from the mutation of the tumor suppressor genes or proto-oncogenes [17]. Cancer is the second leading cause of death globally, with 18.1 million new cancer cases in 2018, and expected to reach 29.4 million by 2040 [18]. As per the World Health Organization (WHO) latest report, 70% of total death due to cancer happen in low and middle-income countries [19]. Cancer is considered a multifactorial disease with genetic defects, including lack of DNA repair or mutation at the time of DNA replication, which contributes to approximately 5-10% of the cases, and external factors such as environmental exposure to UV radiation, toxic substances, and infectious agents contributing the majority (90-95%) of the cases of cancers [20, 21]. Cancer types such as stomach, colorectal, lung, liver, breast, and prostate have a major share in mortality. Cancer cells demonstrate various altered physiological activities, such as apoptosis resistance, insensitivity to growth, growth-inhibiting signals, metastasis, and unlimited proliferation with sustained angiogenesis [21]. Out of various options available for cancer treatment, chemotherapy with drugs having cytotoxic and immunotoxicity seems to be the best option in the present scenario.

Lung cancer is the deadliest and most frequently occurring malignancy with the leading cause of mortality worldwide. Like other cancers, lung cancer involves proliferation, metastasis and invasion [22]. If lung cancer is detected at an early stage, surgery is the best possible solution. However, in most cases, it is detected at the advanced stage. In this scenario, the majority of the patients necessitate systemic chemotherapy. However, systemic chemotherapy frequently leads to side effects such as diarrhoea, nausea, and vomiting. The former may be the result of the changes in the intestinal flora, intestinal barrier dysfunction, and epithelial cell apoptosis of the intestine [23]. Hence, there is a need for additional treatment options, such as maintaining the microbiome population in the lungs and balancing it with the gut microbiome [24].

There are two broad classes of lung cancers such as small-cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Out of these two types,

CHAPTER 11

Probiotics-based Anticancer Immunity in Head and Neck Cancer

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Abstract: Every day we are used to hearing about cancer and its effects. Head and neck cancer is one of the types of cancer which is leading to mortality. Treatment of cancer is crucial to lead a happy and healthy life. Till today several medical strategies, such as radiotherapy, chemotherapy, *etc.*, have come forward to eradicate cancer, but along with these approaches, probiotics are also taking part to dissolve this problem. In simple words, probiotics are microorganisms that are present in fermented foods like yogurt, cheese, creams, fermented milk, *etc.*, which, when administered to the host, provide health benefits. Some familiar probiotics are *Lactobacillus bulgaricus*, *L. casei* and *Streptococcus thermophilus,* which are involved in cancer treatment. Much evidence has proven its health benefits. This chapter focuses on how probiotics act on cancer cells with an introduction to head and neck cancer, thereby triggering our interest to probe into further research on treating cancer using probiotics.

Keywords: Chemotherapy, DNA damage, Head and neck cancer, *Lactobacillus bulgaricusL. casei*, Radiotherapy, Probiotics, , *Streptococcus thermophilus*.

1. INTRODUCTION

Cancer has become more prominent in every nook and corner of the world. “Head and neck cancer” includes distinct kinds of tumors which metastasise in or around the mouth, nose, and throat [1]. Comparatively, men are 2-3 times more affected by head and neck cancer than women. Among all cancers, head and neck cancer is the 6th prominent cancer, accounting for 500,000 cases every year [2]. The use of alcohol, cigarettes and human papillomavirus (HPV) infection is thought to be the leading causes of head and neck cancer.

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Mitesh Kumar Dwivedi, Alwarappan Sankaranarayanan & Sanjay Tiwari (Eds.)

The most prevalent type of head and neck cancer is squamous cell carcinoma (SCC) [3]. The SCC begins in the squamous cells, which are thin, flattened cells that cover the outermost layer of the skin. Squamous cells are found in the lungs, mucous membranes, digestive tract, and urinary tract, in addition to the skin. SCC of the skin is known as cutaneous squamous cell carcinoma (cSCC). This transpires due to the mutation in squamous cell's DNA, resulting in unregulated cell division [4]. People under the age of 40 or above are more prone to this cancer. When it comes to the matter of cure, all types of head and neck cancers are curable, if they are found early. However, after a certain stage, they can be treatable but not curable. The life expectancy for the head and neck cancer patients is between 1-5 years after diagnosis, through the common cancer treatments, which include surgery, chemotherapy, radiotherapy, immunotherapy, targeted therapy, *etc.* However, the recent strategy, which uses probiotics, has gained more attention than the other cancer treatments, which use live bacteria delivered through food and capsules.

Probiotics are live bacteria that give health advantages, when supplemented through yoghurt, milk, cheese, creams, *etc.*, according to the world health organization (WHO) [5]. The most prevalent probiotics present in meals are lactic acid-producing bacteria. Probiotics have long been known to have a wide range of health advantages in both humans and livestock. They aid in the protection of the host against hazardous bacteria as well as the immune system's strengthening. Probiotics have also been shown to aid in metabolic issues and digestion problems. Although the exact methods by which probiotic bacteria provide health advantages are uncertain, they could include competitive exclusion of enteric pathogens, production of antimicrobial metabolites, neutralization of dietary carcinogens and activation of the immune system [6]. The current chapter discusses the types of head and neck cancer, the role of probiotics in cancer treatment, the mode of delivery and the challenges in using probiotics to treat head and neck cancer.

2. TYPES OF HEAD AND NECK CANCER

Head and neck malignancies are a major concern in our nation, accounting for around one-third of all cancer cases [7]. Head and neck cancer includes different types of cancer which are widely spread with common risk factors, namely, alcohol, tobacco and HPV infection [8]. In certain parts of India and Southeast Asia, the practice of blending tobacco with betel nuts has been related to head and neck squamous cell carcinoma (HNSCC). More than 200 million individuals are thought to padlock in this practice around the world, coming to a 2.8-times higher hazard of creating HNSCC, and this increments to more than 10 times when smoking is additionally practiced [9]. Tobacco products have been around for

millennia, but only in the last 60-70 years, we have gained a better knowledge of their harmful consequences (Table. **1**). This new knowledge has paved the way for educational and regulatory initiatives targeted at lowering cigarette consumption. Many carcinogens are thought to damage DNA structure by forming DNA adducts, which is thought to be a frequent mutagenesis route. Moreover, oxidative-induced lesions and damages occur, in protein cross-linked by carcinogens [10]. The different types of head and neck cancer are presented in Table. **1**.

Table. (1). Different types of head and neck cancer, their symptoms and associated genes.

Type of Cancer	Area Affected	Gene Involved	Symptoms	Reference
Oropharyngeal cancer	Oropharynx	TP53	Sore throat, trouble in swallowing, ear pain, & lump in the back of mouth or throat	[11] [12]
Hypopharyngeal cancer	Hypopharynx (bottom part of the throat)	p16	Change in voice, hoarseness, & trouble in breathing	[13] [14]
Laryngeal cancer	Larynx	p16	Hoarseness, & pain while swallowing	[15] [16]
Lip and Oral cavity cancer	Any part of the mouth	TP53	Chronic sore throat, swelling of lips, gums, cheek, & patches in the mouth, & bleeding in the mouth	[12] [17]
Nasopharyngeal cancer	Nasopharynx (upper part of the throat)	MST1R	Lump in the neck, blood in saliva, nasal congestion, headache, & frequent ear infections	[18] [19]
Paranasal sinus and Nasal cavity cancer	Any of the sinuses	-	Nasal discharge, & bleeding from the nose	[20]
Salivary gland cancer	Any of the salivary gland	AR	Difficulty in opening mouth, weak facial muscles on one side of face, persistent pain in salivary gland, & rapid tumour growth	[21] [22] [23]
Squamous cell neck cancer	Outermost surface of the skin	P53	Firm red nodule, ulcer, scaly patch inside the mouth, & itching	[24] [25]
Soft tissue sarcoma	Soft tissues	PTCH1	Noticeable lump, & pain	[26] [27]
Thyroid cancer	Thyroid cells	RAS, BRAF	Lump in the neck, change in voice, difficulty in swallowing, & pain in the throat.	[28] [29]

[**Abbreviations**: TP53, tumour suppressor gene; MST1R, macrophage stimulating 1 receptor; AR, androgen receptor; PTCH1, protein patched homolog; RAS, Rat sarcoma virus; BRAF, B-Raf proto-oncogene serine / threonine-protein kinase]

SUBJECT INDEX

A

B

C

D

E

M

N

P

R

S

T

U

V

W

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