Neeraj Mishra · Shvetank Bhatt ·
Keshav Raj Paudel · Philip M Hansbro ·
Kamal Dua *Editors*

Synbiotics for the Management of Cancer

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Preface

Cancer is a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020, or nearly one in six deaths. Breast, lung, colon, and rectum, and prostate cancers are the most common cancers. Most of the cancer cases are due to tobacco use, high body mass index, alcohol consumption, less intake of vegetables and fruits, and less physical activity. Many cancers are treated successfully if detected in early stages. The drawback of this deadly disease is that generally it is detected only in the later stage. The present challenges faced by the treatment of cancer are mainly due to the heterogenicity of some cancers, drug resistance, late diagnosis, few treatment advances for early-stage cancer, non-selectivity of drugs towards cancer cell leading to side effects and many more which are still in the dark.

Synbiotics is the combination of pre- and probiotics. Synbiotics has predominant role in effective treatment of various diseases including cardiovascular, reproductive, metabolic, neurodegenerative, gastrointestinal, thrombotic, skin, inflammatory disorders, and cancer. In recent times, synbiotics are emerged as a potential therapeutic approach for the treatment of various cancers. The risk of getting affected with cancer can be reduced using synbiotics. Use of synbiotics has great influence on the number of beneficial microbes. These microbiotas are having important role in the pathophysiology of cancer. In addition to producing an effect on the growth of tumors, they also have the ability to modify the microenvironment of cancer cells. These microbes also manipulate the oxidative stress, immune response, and inflammatory response of the cell.

The available treatment of cancer such as chemotherapy, radiation, and targeted therapy are associated with lots of adverse effects and some of them are severe in nature. The use of synbiotics may reduce these adverse effects and improve the quality of life of cancer patients. Moreover, cancer chemotherapy and radiations therapy are linked with severe pain and inflammation. Use of pre- and probiotics in combination with other agents can reduce the severity of above symptoms. The manipulation of different axis like brain-gut-microbiota and lung-gut-microbiota

may be considered as an important approach prevention and treatment of cancer. However, still there are a lot of investigations underway.

We hope the book will be a useful compilation for undergraduate, postgraduate, and doctoral students and also for the translational and clinical researchers working in the field of cancer and drug delivery, research, and development.

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Sydney, NSW, Australia — Keshav Raj Paudel
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We would also like to express our sincere gratitude to Honorable Founder-President Dr. Vishwanath Karad, MIT World Peace University (MITWPU), Pune, India; Executive President Mr. Rahul V. Karad, MITWPU, Pune, India; Vice Chancellor Dr. R.M. Chitnis, MITWPU, Pune, India; Pro-Vice Chancellor Dr. Milind Pande, MITWPU, Pune, India for their continuous motivation. My sincere thanks to Dr. Neeraj Mahindroo, Dean, School of Pharmacy, MITWPU, Pune, India for his continuous support during the writing of this book. Finally, we would like to express our gratitude to the faculty members of School of Pharmacy, MITWPU, Pune, India.

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Neeraj Mishra works as Professor in Amity Institute of Pharmacy, Amity University Madhya Pradesh, Gwalior, since July 2019. He has done his M. Pharm and Ph.D. in Pharmaceutics from Dr. H.S. Gour Central University, Sagar (M.P.), India. He has around 20 years of teaching and research experience. He has around 100 publications of international and national repute in recent concepts of novel drug delivery system, localized drug delivery, targeted and controlled drug delivery of nanocarriers/microparticles for treatment of breast, colon cancer, and neurodegenerative disorders. Dr. Mishra has a total citation of 2750; h-index 25, and i-index 32. The total cumulative Impact Factor of his published papers is around 100 (as per SCOPUS). He has also written five books and nine book chapters with national and international publishers. He has also published three international and three national patents. Dr. Mishra has also received **"Distinguished Professor Award**" 2019, from DST-SPAICS in the workshop held at Smriti College of Pharmaceutical Education and Research, M.P., India. Recently, Dr. Mishra has received "**Distinguished Professor Award**" 2021 from IPA, MP State Branch, Indore. He also served as **Guest Editor of a thematic issue entitled "Nanoparticulate Carriers for the Treatment of Infectious Diseases and Cancer," Current Molecular Pharmacology, Volume 14, 2021, Issue number 3, Bentham Sciences, USA**.

Shvetank Bhatt currently works as an Associate Professor in the School of Pharmacy, Dr. Vishwanath Karad MIT World Peace University, Pune, Maharashtra, India. He has done M. Pharm in Pharmacology from Manipal College of Pharmaceutical Sciences, MAHE, Manipal, Karnataka, and Ph.D. in Neuropharmacology from Birla Institute of Technology & Science (BITS) Pilani, Pilani Campus, Rajasthan. He has a total of 16 years of industrial and academic research experience. His areas of specialization include CNS disorders, pain, inflammation, and immunooncology. He is a recipient of Prof. Duggirala Visweswaram & Prof. Sreemantalu Satyanarayana Award (Best Paper in Pharmacology-IJPER) in 2012 and 2014. He has published more than 80 papers in various journals of national and international repute. Dr. Bhatt has a total citation of 750; h-index 14, and i-index 24. The total cumulative Impact Factor of his published papers is around 140 (as per

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Keshav Raj Paudel is an IASLC early career researcher at the Centre for Inflammation, Centenary Institute/UTS. He completed his Ph.D. (2017) from Mokpo National University, South Korea, and started his first postdoctoral research at the University of Texas Health Science Centre at Houston Texas, USA, in 2018 on an NIH-funded project. He is an emerging scientist with a good scientific track record demonstrated through his publications and successful grants. After joining UTS in 2019 for a second postdoctoral position, his research trajectory is continuously upraising in the field of translational research with a major focus on pulmonary diseases. As an early career researcher, he is currently leading various in vitro and in vivo projects under the supervision of Prof. Philip Hansbro with his research expertise, to investigate the potential of nanotechnology including IASLC ECR fellowship-2019 (lead CI), TSANZ-grant-in-aid-2021 (lead CI), SPHERE seed grant-2020 (co-CI), Triple I-seed grant (co-CI), and UTS seed grant-2021 (co-CI). Since 2015, he has published more than 100 peer-reviewed scientific articles and book chapters (>50% as first/senior author in leading journals such as *European Respiratory Journal*, *Cell Death and Diseases*, *Cell Reports*, *Journal of Controlled Release*, *Medical Journal of Australia*, *Drug Discovery Today*, and *Antioxidants*), with a total citation of >1800. He is also a journal reviewer of more than 50 journals and has already reviewed more than 140 research papers. As a journal editor, he has handled more than 40 papers so far from *Frontiers in Medicine*, *Frontiers in Pharmacology*, *Antioxidants*, *Journal of Immunology Research*, *Oxidative Medicine and Cellular Longevity*, and *Heliyon*.

Philip M Hansbro is Director, Centre for inflammation, Centenary Institute/UTS, NHMRC Level 2 Investigator & Conjoint Professor in Research Centre for Healthy Lungs, Hunter Medical Research Institute/University of Newcastle. He built and leads an internationally renowned group that investigates the pathogenesis of respiratory diseases (asthma, COPD, IPF, respiratory infections, lung cancer). He has extensive expertise in developing/using mouse models that recapitulate the major features of human disease and state-of-the-art mouse research facilities. He has published >400 publications in high impact journals: ~4000 citations/year.

Kamal Dua is a Senior Lecturer in the Discipline of Pharmacy at the Graduate School of Health, University of Technology Sydney (UTS), Australia. He has research experience of over 12 years in the field of drug delivery systems targeting inflammatory diseases. Dr. Dua is also a Node Leader of Drug Delivery Research in the Centre for Inflammation at Centenary Institute/UTS, where the targets identified from the research projects are pursued to develop novel formulations as the first step towards translation into clinics. Dr. Dua conducts research in two complementary areas: drug delivery and immunology, specifically addressing how these disciplines can advance one another, helping the community to live longer and healthier. This is evidenced by his extensive publication record in reputed journals. Dr. Dua's research

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Introduction to Cancer Genetics and Its Symbiotic Relationship

1

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1.1 Introduction

The second-leading cause of mortality in the US is cancer, which is a significant global public health concern that might affect everyone. Cancer diagnosis, identification, and treatment were negatively impacted by the coronavirus disease 2019 (COVID-19) pandemic in 2020. Compromised access to clinical care because of COVID-19 caused delays in the diagnosis and treatment of cancer. Consequently, an increase in advanced-stage illness, ultimately resulting in a greater death rate was recorded reflecting a false short-term drop in the incidence of cancer. Both traditional and nanotechnological methods of cancer therapy are effective. The conventional techniques currently applied for the treatment of different stages of cancer are radiation, surgery, and chemotherapy (Gautam et al. 2021; Gautam et al. 2019). These are effective when cancer is diagnosed especially in its early stages. In chemotherapy, a higher dose is given which has major side effects which include loss of hair, immunity, weight, etc. Attempts were, however, made by Shrivastava et al. to reduce the drug dose and hence the toxicity while maintaining the therapeutic index to an optimum level (Shrivastava et al. 2020). The targeting carriers are able to carry the drug payload directly to the effective site and could treat cancer effectively. These nanocarriers include liposome (Mukherjee et al. 2022), vesosome (Gautam et al. 2021), noisome (Haroun et al. 2022), solid lipid nanoparticles (Wei et al. 2022), emulsion (Youssry et al. 2022), micelles (Sun et al. 2022), polymeric nanoparticles (Bhattacharya et al. 2022), dendrimer (Fatima et al. 2022), nanocomposite (Sharma

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et al. 2018), carbon nanotubes (Bura et al. 2022), mesospheres (Gautam et al. 2020), etc. In addition, different herbal extracts are also combined with synthetic anticancer agents are also included in the treatment of cancer and used as combination chemotherapy. Some of the examples are sulphoraphane, taxol derivatives (paclitaxel, docetaxel), epipodophyllotoxin, vinca alkaloids (vincristine, vinblastine, vindesine, vinflunine, vinorelbine, etc.), pomiferin, roscovitine, etc. (Gautam et al. 2022; Greenwell and Rahman 2015).

1.1.1 Synbiotic of Cancer

The importance of the human microbiome in cancer pathogenesis is becoming better recognized. Prebiotics, probiotics, and synbiotics are a few of the well-researched methods for altering the microbiota for therapeutic gain, and interest has been drawn to their possible therapeutic role in cancer therapy and prevention. The human microbiome participates in carcinogenesis as a key partner in a tripartite "interactome" between the host and the environment. Prebiotics, probiotics, and synbiotics may have impacts on metabolism, intestinal barrier function, immunomodulation, and antiproliferative effects, among other potential anticarcinogenic pathways. In Fig. 1.1, it is demonstrated how cancer can be cured by the use of synbiotics. The Bacillus Calmette-Guerin system has been explored to treat superficial bladder cancer. Probiotics have been demonstrated to have direct anticancer benefits in a variety of mouse models, and researchers are investigating genetically modified microorganisms to boost their anticancer activity or to use them as delivery systems for effective chemotherapy. In human cancer, the primary role of microbiome modifying therapy has been appreciated as an adjuvant component(s), which enhances the efficacy of radiation as well as chemotherapy, antibiotics, and surgery while minimizing their side effects. The healthy gut microbiome has a varied microbial population as vital components, and current research indicates that this community may also affect chemotherapy response and overall cancer survival. Some examples of synbiotics are expected to reduce/decrease the side effects of conventional chemotherapy.

B. animals and amylose corn starch enhance the growth of bifidobacteria which are present in most food products. *B. longum* and fructooligosaccharide (FOS) are present in curd, and help in the growth of *L. acidophilus*, *L. rhamnosus GG*, *L. casei Shirota*, and *Bifidobacterium*, also impede the growth of cancer cells. In addition to boosting the immune system, they also reduce the levels of fecal enzymes. These enzymes catalyze the formation of cancer-causing amines and inhibit bacteria involved in the conversion of procarcinogens to carcinogens. *L. acidophilus and Bifidobacterium species* reduced the peptic ulcer, nonulcer dyspepsia, gastro-oesophageal and gastric cancer (Yadav et al. 2022).

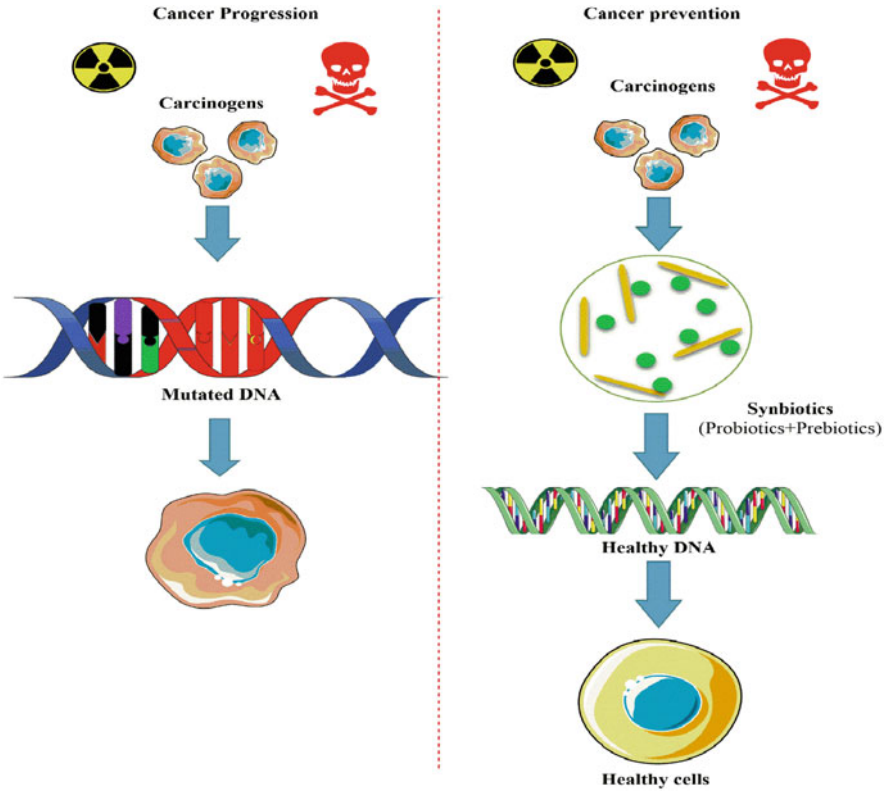

Fig. 1.1 Role of synbiotics in cancer

1.2 Genetics of Cancer, and the Role of Oncogenes in Signal Transduction

Cancer is caused by various factors including the environment (pollution), harmful substances (aflatoxins, asbestos, vinyl chloride, etc.), viruses (hepatitis-B, human herpes virus-8, human papilloma, and Epstein–Barr), lifestyle (nutrition, physical activity, tobacco), epigenetics, and genetic factors (Allahverdi et al. 2021). On a genetic level, oncogene activation, tumor suppressor gene inhibition, and mRNA production all contribute to the development of cancer. Genes are necessary for normal cell proliferation and differentiation; however, their erroneous expression leads to inappropriate cell proliferation (Barbato et al. 2017). Oncogene activation involves the introduction of epigenetic changes into cellular protooncogenes. The cell gains a growth advantage as a result of these genetic changes. The three genetic pathways that activate oncogenes in human malignancies are chromosomal rearrangements, mutations, and gene amplifications. These mechanisms either alter the structure of protooncogenes or increase protooncogene expression. As

tumorigenesis is a multistep process, a number of these mechanisms frequently contribute to human tumorigenesis (conversion of gene fragments related to cancer). The ability to spread is usually achieved through a combination of protooncogene activation and loss or inactivation of tumor suppressor genes (Pierotti et al. 2000). Most tumors progressively turned into cancer because of spontaneously/inherited changes in the genetics of cellular genes; such as DNA damage caused by carcinogens present in the environment or by replication error during the mutation (Sever and Brugge 2015). Naturally occurring mutations accumulate in somatic cells during life. Many of these mutations have no noticeable impact, but a few can change important biological processes. Early somatic mutations can cause developmental issues, however, the steady accumulation of mutations over the course of a lifetime can result in cancer and accelerate the aging (Martincorena and Campbell 2015). These gain-of-functional mutations lead to the formation of oncogenes and give the cells a selective advantage, which, when combined with changes in the microenvironment, promotes tumor growth and progression (Sever and Brugge 2015). Mutations occur as a result of replication errors or DNA damage which is either incorrectly repaired or left unrepaired. Exogenous and endogenous factors are responsible for mutations. Ionizing radiation, toxins, and UV light are examples of exogenous agents that can harm DNA. On the contrary, endogenous factors including, among others, enzymes involved in DNA repair or genome editing, aldehydes, mitotic mistakes, and reactive oxygen species (Lynch 2010) may equally be responsible for carcinogenesis. Base substitutions, deletions, and insertions are examples of mutations that can activate protooncogenes. These mutations lead to the activation of protooncogenes by structural modifications in their encoded proteins. Changes that are typically involved in critical protein regulatory regions often result in the uncontrolled and persistent activity of the mutant protein (Bishop 1991). Oncogenes, i.e., RAS, BRAF, β-catenin, and Myc are primarily protein-coding genes whose functions are controlled by their gene products-proteins (Yu and Li 2015). RAS (H-ras, K-ras, and N-ras) has long been a major focus of cancer research because due to its frequent mutation in many human tumors. Mutated or oncogenic RAS abnormally initiates a network of interconnected signaling pathways, including protein kinase C (PKC), phosphoinositide-3 kinase (PI3K)/AKT pathways, mitogen-activated protein kinases (MAPK), and Ral guanine nucleotide dissociation stimulator (RalGDS), among others (Khan et al. 2019). Its function is to transform the extracellular environment the signal(s)/changes into a cell's internal chain (signal transduction). RAS mutant protein controls angiogenesis, metabolism, apoptosis, and tumor cell proliferation through downstream signaling pathways (Chen et al. 2019). RAS proteins alternate between an inactive GDP-bound and an active GTP-bound conformation. Guanine nucleotide exchange factors (GEF) promote activation, while GTPase-activating proteins promote inactivating GTP hydrolysis (GAP). Ras may interact with more than 20 distinct proteins from 10 different effector families as a result of a conformational change brought about on Ras activation. Effector proteins are concentrated by activated RAS into plasma membrane signaling nanoclusters, where they engage in interactions with necessary lipids and proteins to regulate downstream pathways. Cancer frequently harbors RAS

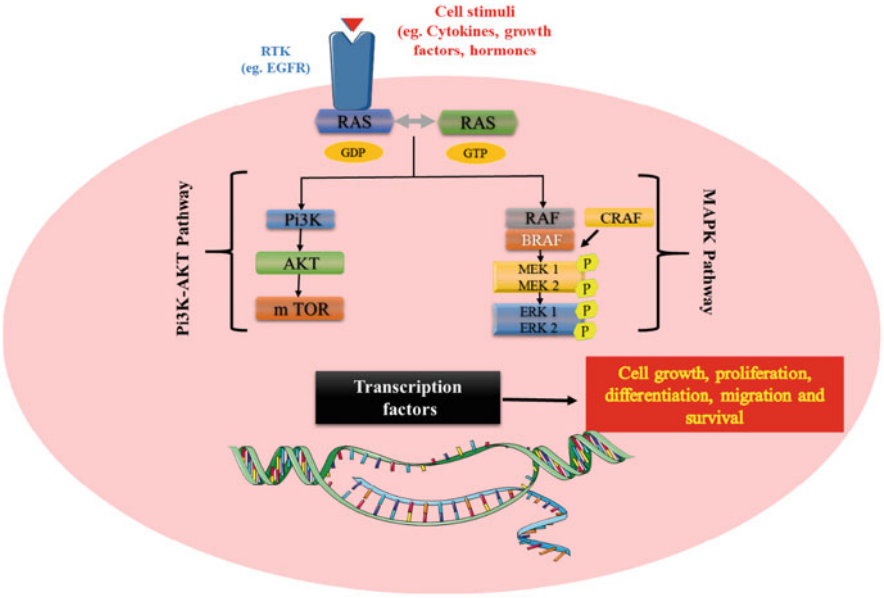

Fig. 1.2 RAS-mediated signaling pathways

mutations that make the protein constitutively active. Although the mutation rates linked to each RAS gene and department on the type of cancer and accordingly exhibit unique patterns (Prior et al. 2020). BRAF genes, which encode a serine/threonine kinase that is a component of the RAS-RAF-MEK-ERK axis, which regulates cellular growth, are susceptible to another sort of mutation (Ducreux et al. 2019). The BRAF kinase domain is exclusively encoded by amino acids 457–717. Residues 596–600 are home to the kinase activation loop, which collaborates with the phosphate-binding loop to keep the kinase engaged. BRAF can phosphorylate and activates the mitogen-activated kinases 1 and 2 (MAP 2K 1/2) signaling pathway by phosphorylating the tyrosine and threonine residues of the MAPK ERK1/2 proteins. Vimentin and keratin-8, as well as other members of the MAPKAPKK family of cytoskeletal proteins, are phosphorylated to activate ERK1/2. ERK 1 and 2 will also translocated into the nucleus, where they will activate transcription factors like FOS, TP53, and ELK1 (Fig. 1.2) (Alvarez and Otterson 2019; Pritchard and Hayward 2013).

Furthermore, oncogenic activation of PI3K, AKT, and mTOR pathways constitutes a common event in prostate cancer and promotes tumor formation, disease progression, and therapeutic resistance (Shorning et al. 2020). The PI3K pathway contains several key nodes such as AKT and mTOR that play critical roles in this pathway resulting in a wide array of functional outcomes including cell survival, growth, and differentiation. Ligand receptor interaction involved in which insulin-like growth factor receptor interacts with insulin and activates the channels, which intern activates PI3K. Activated PI3K catalyzes the

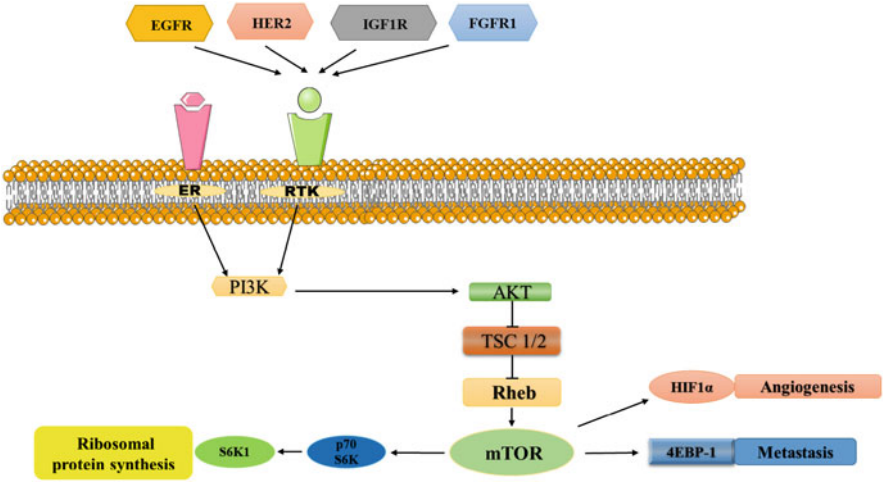

Fig. 1.3 Progression of cancer through receptor-mediated signaling of PI3K/Akt/mTOR resultant angiogenesis and metastasis

phosphorylation of PIP2 at position 3 of the inositol ring to produce PIP3, which recruits AKT and PDK1 to the plasma membrane via their PH domains. mTORC2 phosphorylates the AKT on Ser473, changing its conformation and allowing PDK1 to phosphorylate it on Thr308 once it has been recruited to the cell membrane. Proteins from the cell membrane are phosphorylated by activated AKT, which then loses its attachment to the membrane and phosphorylates other target proteins in the cytosol and nucleus. Target protein phosphorylation promotes cell proliferation, growth, and survival. Figure 1.3 showed the signaling pathway involved in the process of angiogenesis and metastasis (Lim et al. 2014; Miricescu et al. 2020).

β-Catenin is a multifunctional protein that has a role in both cell adhesion and transcription. It serves as an important coactivator downstream of the oncogenic Wnt signaling pathway. Endometrial, liver, and colorectal cancers have all been related to mutations in the β-catenin gene (CTNNB1) (Kim and Jeong 2019). β-catenin-dependent and independent intracellular signaling pathways can both be activated by the cysteine-rich glycoprotein family known as Wnt proteins (Skronska-Wasek et al. 2018). According to the report β-Catenin, the dependent signaling pathway is activated by the binding of the Wnt ligand to the LRP-5/6 receptors (low-density lipoprotein receptors) and Frizzled receptors. After their activation Disheveled (DVL), causes the complex (Axin, GSK-3 beta, CK1, APC) to be recruited to the receptor (Singla et al. 2020). The Wnt-Frizzled-Axin-LRP-5/6 complex binds to cytosolic GSK-3 beta, rendering it incapable of phosphorylating beta-catenin. Unphosphorylated β-catenin accumulates in the cytosol and migrates to the nucleus, where it interacts with TCF/LEF and coactivators such as Pygopus (Pygo) and Bcl-9 to activate Wnt target genes such as c-Myc, cyclin D1, and Cdkn1a which promote and activate many developmental, cancer-related, and pathogenic genes (Nusse and Clevers 2017; Krishnamurthy and Kurzrock 2018).

1.3 Tumor Microenvironment

Cancers are complex "rogue" organs that recruit several additional cells and can be corrupted by the altered cells; they are not just collections of malignant cells (Tarin 2012). Malignant and non-transformed cell interactions result in the tumor microenvironment (TME) (Kurelac et al. 2020). At all stages of carcinogenesis, the TME's non-malignant cells have a dynamic and often tumor-promoting activity. An intricate and dynamic network of growth factors, chemokines, cytokines, inflammatory, and matrix remodeling enzymes supports intercellular communication against a background of significant alterations in the tissues' physical and chemical properties (Rowley et al. 2019). The evolution, shape, and activity of TME cells share many similarities with wound healing and inflammation processes although cells like macrophages are also present in malignancies that have no known link with chronic inflammatory disorders. One reason for this is that oncogenic alterations in malignant cells increase inflammatory and wound-healing pathways (Balkwill et al. 2012).

The significance of the microenvironment in the beginning and progression of cancer is recognized as crucial for improved molecular diagnostics and therapies. The tumor microenvironment is the result of cell-to-cell communication (Baghban et al. 2020). In epithelial malignancies, for example, these cells include invasive carcinoma and its stromal elements. Tumor-associated fibroblasts, for example, offer an important communication network in the microenvironment by secreting growth factors and chemokines, altering the ECM, and therefore delivering additional oncogenic signals that promote cancer cell proliferation and invasion (Hu et al. 2022). It has been established that tumor-associated stromal cells play an active role in cancer progression. The ECM, fibroblasts of various phenotypes, and a framework of immunological and inflammatory cells, blood and lymph arteries, and nerves make up stromal elements (Liu et al. 2021). The present understanding of the TME is that it has a significant impact on tumor progression and that altering its characteristics could provide unanticipated therapeutic effects.

Tumor cells, like all other cells in the body, require nutrition, gas exchange, and metabolite removal in order to proliferate (Deberardinis and Cheng 2010). This contribution comes via circulation through blood vessels, which also act as the entry and exit locations for immunological and other circulating cells from the bone marrow. Tumors have a high rate of angiogenesis due to the high nutritional and oxygen requirements of tumor cells, which enables them to continue growing at a constant rate (Eales et al. 2016). For tissue homeostasis and growth, establishing a functioning vascular network is critical. The balance of growth factors and diverse vascular and nonvascular cell components is critical for the blood vessels, which constitute a complicated network (Franco et al. 2020). It is vital to remember that these variables become unbalanced in cancer, allowing for the development of physically and functionally distinct tumor vasculature. Aside from neoplastic cells, tumors are made up of a variety of components. Cells including fibroblasts and endothelial cells, as well as immune cells, are among the non-cancerous components (Bae et al. 2018; Franco et al. 2020). This group of cells is known as the tumor stroma, and it is a part of the tumor microenvironment (Li et al. 2007). The

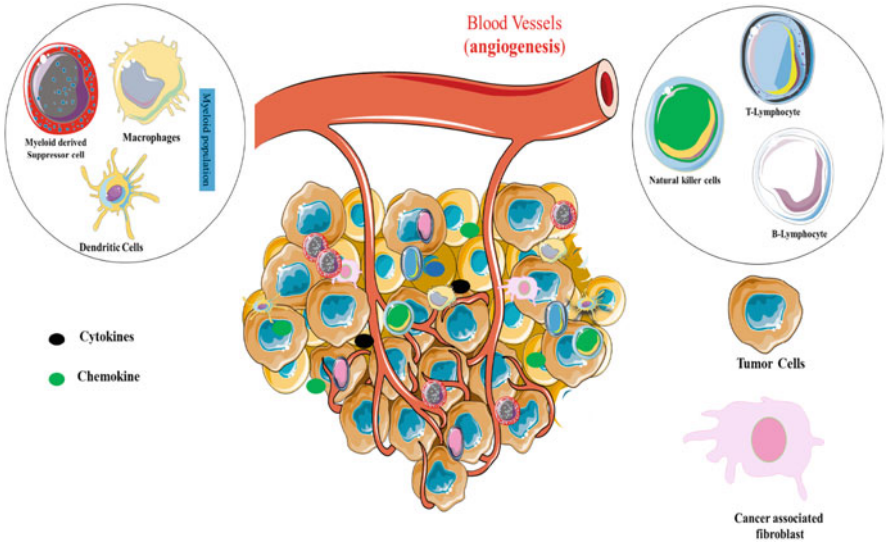

Fig. 1.4 The components of TME. Involvement of extracellular and cellular components in TME. Cellular components include various hematopoietic cells (tumor-associated macrophages, B-cells, T-cells, DCs, NK cells, etc.), and resident stromal cells (cancer-associated fibroblast cells, endothelial cells, etc.). Extracellular components include ECM and cell-secreted proteins like cytokines and growth factors

microenvironment is important in several aspects of carcinogenesis, including the formation of tumor vasculature, which is important in the progression of cancer to metastasis (Arneth 2019). More recently, it has been discovered that the tumor's microenvironment influences treatment response. Furthermore, tumor stroma manipulation can improve the efficacy of current medicines and open up new avenues for therapeutic targeting (Sun 2016). The primary components and stromal cells are linked to immune system cancer cells, vascular system capillaries, and cells, mesenchymal support cells including adipocytes and fibroblasts and the ECM which surround cancer cells. Cytokines, chemokines, growth factors, and antibodies are all found in the stroma, in addition to cells (Anderson and Simon 2020). Figure 1.4 shows structural feature of the microenvironment of tumor.

1.3.1 Cells of the Tumor Microenvironment

The TME contains tumor vasculature, immune cells, fibroblasts, lymphatics, pericytes, and occasionally adipocytes in addition to malignant cells; all of these components are discussed in more detail below. To distinguish these cells, cell-type-specific markers, which are often cell surface molecules, are routinely utilized (Roma-Rodrigues et al. 2019; Anderson and Simon 2020).

1.3.1.1 T-Lymphocytes

The invasive tumor boundary, various T cell populations, and lymphoid organs are all infiltrated by T cells inside the TME. One of them, antigen-experienced cytotoxic CD8+ memory T cell (CD8+CD45RO+), is highly correlated with a favorable prognosis and are capable of eradicating tumor cells (Pruneri et al. 2018). The CD4+ T helper 1 (TH1) cells that generate the cytokines interleukin-2 (IL-2) and interferon-gamma (IFN-γ), which are linked to a good prognosis, stimulate CD8+ T cells. Other CD4+ cell populations, such as TH2 cells produce IL-4, IL-5, and IL-13 to support B cell responses. On the other side, TH17 cells produce IL-17F, IL-17A, IL-21, and IL-22 which encourage antimicrobial tissue inflammation and tumor development. TH2 cells in breast cancer and the TH17 cells in esophageal tumors are linked to positive results (Ouyang et al. 2008). The immunosuppressive T regulatory cells (Tregs), which can be identified by the expression of FOXP3 and CD25, are the CD4+ T cells (tumor-promoting cells) (Liu et al. 2016). The synthesis of IL-10, TGF-β, and cell-mediated interaction via cytotoxic T-lymphocyte antigen 4 (CTLA4), leads to Tregs-mediated suppressive immune response. This prevents the identification of tumor cells and increases the immune response. In many forms of cancer, a high number of Tregs in the TME is associated with a poor prognosis. As found in numerous B cell cancers, Tregs can also be tumor suppressive. Their presence in Hodgkin's lymphoma is associated with a favorable prognosis, perhaps as a result of indirect tumor cell growth inhibition. γδT lymphocytes have potent cytotoxic activity against a variety of cancerous cells including cancer stem cells and resemble innate immune cells rather than adaptive immune cells in several ways (Zou et al. 2017). It is unclear if the presence of γδT cells in the TME indicates a good or negative prognosis, despite experimental animal cancer research suggesting they serve as immune surveillance cells (Lo Presti et al. 2020).

1.3.1.2 B Lymphocytes

The lymph nodes and lymphoid structures that discharge into the TME are where B cells are most prevalent, however, they can also be detected close to the tumor's invasive edges. In some breast and ovarian malignancies, having B cells infiltrate the TME is attributed to a better prognosis; yet, in mice models, tumor-specific cytotoxic T cell responses are blocked by B cells (Tsou et al. 2016; Sharonov et al. 2020). Newer data demonstrate that B cells and immunoglobulin deposition promote tumors in a recurrent animal study of skin cancer (Karagiannis et al. 2012). An IL-10-deficient population that suppresses the immune system B cells that produce regulatory B cells (Bregs) or B10 cells (Floudas et al. 2016). One of the studies done by Mauri and Bosma, in which they said enhances tumor volume and inhibits immunogenicity peculiar to malignancies in skin cancer brought over by aggravation. Additionally, they show a preference for lung metastasis in a mouse model (Mauri and Bosma 2012). Breast cancer is a disease that affects women. Bregs also obstruct tumor clearance. Anti-CD20 antibodies were used to kill cells in a lymphoma mouse model. However, none of these outcomes seem to be brought on by Bregs invading the TME; rather, they seem to be brought on by Bregs influencing other immune cells in the neighboring lymphatic tissue or emptying lymph node, as

well as altering myeloid cell activity (Michaud et al. 2021). It is unclear whether B cells and Bregs, in particular, play similar functions in human malignancies (Gupta et al. 2019).

1.3.1.3 Natural Killer and Natural Killer T-Cells

Natural killer (NK) and natural killer T (NKT) cells, which are intrinsic cytotoxic lymphocytes, invade the tumor stroma without colliding with tumor cells (Balato et al. 2009). They appear to predict a positive prognosis for numerous malignancies, including colorectal, gastric, lung, renal, and liver tumors. NK cells, despite their presence in the TME, may not be able to carry out their tumor-killing activity. Several investigations have shown that transforming growth factor-beta (TGF-β) generated from malignant cells activates the anergic phenotype of NK cells in the tumor stroma (Fridman et al. 2012).

1.3.2 Tumor-Associated Microphages

Several human and clinical mouse malignancies are attributed to the presence of large numbers of TAMs, which usually behave in a pro-tumorigenic manner (Solinas et al. 2009). According to Condeelis and Pollard's research, TAMs are essential allies for invasion, metastasis, and migration (Condeelis and Pollard 2006). Most TAMs express mannose and scavenger receptor class A and have an IL-10 high, IL-12 low phenotype. An excess of TAMs in the TME has been linked to a poor prognosis in both preclinical and clinical studies (Giakoustidis et al. 2015). Furthermore, follicular lymphoma gene array investigations reveal that, despite certain other prognostic factors, the level of expression connected to a prominent "macrophage" pattern implies a poor prognosis. Lin et al. (2006) and Zumsteg and Christofori (2009) discovered that macrophages are key players in tumor angiogenesis. Transcriptional screening on high-density oligonucleotide probes shows that TAMs are media to enhance an organization in transcriptome encoding angiogenic substances (Mayi et al. 2012; Hasan et al. 2019). Macrophages' phenotype and reactivity to environmental factors are shaped by their bidirectional interaction with the tumor microenvironment (Bhatta and Cooks 2020). Tumor hypoxia is important although many TAMs congregate in hypoxic and/or necrotic areas of tumors. TAMs are considered to be drawn to these areas by hypoxia-induced chemotactic factors such as vascular endothelial growth factor (VEGF), endothelins, and endothelial-monocyte activating polypeptide II (EMAP2). There have been discoveries of unique hypoxia-induced pro-angiogenic phenotypes in human macrophages (Kes et al. 2020).

1.3.3 Myeloid-Derived Suppressor Cells

A group of immune cells known as myeloid-derived suppressor cells (MDSCs) is more prevalent in a variety of rodent and human cancers (Veglia et al. 2018). Human

MDSCs are challenging to characterize since their phenotypic is so diverse. They can even separate themselves into TAMs. Nitric oxide synthase 2 (NOS2) and arginase are produced by murine and human MDSCs, respectively, and these enzymes prevent CD8+ T cell activation (ARG1). They also cause Tregs to grow and macrophages to polarize into a TAM-like phenotype (Ugel et al. 2021).

1.3.4 Dendritic Cells

Dendritic cells are essential for the processing and presenting of antigens (DCs). It is thought that the TME's DCs are defective because they cannot adequately initiate an immune system response to tumor-associated antigens (Lucarini et al. 2021). DCs function to activate immunological function is further hampered by the TME's hypoxic and inflammatory surroundings, and certain DCs have been reported to decrease T cell responses at the tumor site (Paardekooper et al. 2019; Giovanelli et al. 2019). Two recent studies have discovered ZBTB46 as a new transcription factor that is expressed specifically in all conventional human and murine DCs (Anderson et al. 2021). This study provides evidence that DCs belong to a unique immune cell stream, advancing our understanding of DCs in the TME.

1.3.5 Tumor-Associated Neutrophils

Tumor-associated neutrophils (TANs) may or may not contribute to the development and spread of primary tumors (Yuan et al. 2016). In mouse cancer models, neutrophils have been shown to enhance primary tumor growth and have pro-tumorigenic effects by promoting angiogenesis, increasing ECM breakdown, and suppressing the immune system (Singel and Segal 2016). Additionally, CD11b+ bone marrow-derived cells, a polymorphic myeloid cell pool, have been linked to premetastatic lung prepping and an increase in the spawning of circulating tumor cells (Peinado et al. 2017). Following immunological or cytokine activation, however, these cells have been shown to have anticancer activity. In these circumstances, neutrophils can proactively eliminate spreading tumor cells as well as indirectly via TGF-b suppression (Baumann et al. 2022).

1.3.6 Cancer-Associated Fibroblasts

In response to paracrine cues, residential fibroblasts develop into myofibroblasts when tissues are damaged. Myofibroblast stimulation can also lead to organ fibrosis, which increases the risk of cancer development (Piersma et al. 2020). Myofibroblasts, also known as cancer-associated fibroblasts, are common in several TMEs (CAFs). Endothelial cells, smooth muscle cells, myoepithelial cells, mesenchymal stem cells, and others are among the origins of CAFs (Xing et al. 2010). CAFs generate growth factors that are mitogenic for malignant cells, such as

fibroblast growth factor (FGFs), hepatocyte growth factor (FGFs), and insulin-like growth factor 1 (IGF1). Malignant cells undergo the epithelial-mesenchymal transition (EMT), which contributes to the immune-suppressive milieu when TGF-b from fibroblasts is present. By functioning as a chemoattractant and promoting the migration of other stromal cell types and their progenitors into the TME, CXCL12, a chemokine generated by fibroblasts, can both promote the proliferation and survival of malignant cells (Sun et al. 2010; Daniel et al. 2020).

CAFs exhibit a proinflammatory gene profile in mice models of skin, breast, and pancreatic cancers, which aids tumor progression by increasing neovascularization and immune cell recruitment. The tumor-promoting effects are reduced when the transcription factor NF-kB is inhibited, implying that this inflammatory signaling pathway plays a significant role in tumor growth in stromal cells. The architecture of the TME is considerably influenced by fibroblasts' production of ECM components and ECM reformation enzymes (Winkler et al. 2020). Sometimes in malignancies, CAFs are organized into fibrovascular cores that branch out throughout the tumor mass, whereas in others, they surround the malignant cells with compact desmoplastic stroma that may take up the majority of the space and obstruct the delivery of anticancer drugs to the malignant cell target (Barresi et al. 2016). The invasive front of a tumor often has a higher density of CAFs. Researchers recently examined the results of removing tumor-bearing mice's cells that were positive for the fibroblast marker fibroblast activation protein-a (FAP). By eliminating FAP-positive TME cells, which were mediated by IFN-γ and TNF-α, the researchers also showed that these cells are crucial mediators of immune suppression (Zhou 2020).

1.3.7 Adipocytes

In particular malignancies, such as intra-abdominal tumors that metastasize to the omentum, adipocytes actively aid in the recruitment of malignant cells by secreting adipokines and also assist the development of malignant cells by supplying fatty acids as fuel for cancer cells (Balkwill et al. 2012).

1.3.8 Vascular Endothelial Cells

As part of the neovascularization required for the development of cancer, a variety of soluble TME proteins, including VEGFs, FGFs, PDGFs, and chemokines, activate endothelial cells and their supporting pericytes (Carmeliet and Jain 2011). Angiogenesis is activated and new capillaries form from the pre-existing ones when a quiescent blood vessel detects an angiogenic signal from malignant or aggressive cells or as a result of hypoxic conditions in the TME (Carmeliet and Jain 2011; Weis and Cheresh 2011). Almost every feature of the tumor vasculature forms with aberrant function. Blood vessels, for example, are diverse and leaky, with chaotic branching architecture and an uneven vessel lumen. As the arteries leak, the

interstitial fluid pressure increases, resulting in an uneven distribution of blood flow, oxygenation, nourishment, and medicine in the TME. As a result, hypoxia rises, favoring metastasis. Although VEGF (also known as VEGFA), which is generated by both cancerous cells and inflammatory leukocytes, is the key angiogenic factor in the TME, progressed tumors can also generate a range of other angiogenic factors that can take the place of VEGF (Balkwill et al. 2012).

1.3.9 Pericytes

Pericytes are perivascular stromal cells that are an essential part of the tumor's vasculature and provide mechanical stability to the bloodstream. Low pericyte coverage of the vasculature has been linked to a poor prognosis and increased metastases in clinical investigations of bladder and colorectal cancer (Ribeiro and Okamoto 2015). According to a recent study, the relationship between pericyte coverage and negative prognosis may be explained by the fact that decreasing pericytes in mice genetic models led to increased hypoxia, EMT, and MET receptor activation and reduced primary tumor development. In these mice tests, pericyte depletion is also preferable to metastasis. The investigators also observed that invasive breast cancer patients' prognoses were negatively correlated with lower pericyte infiltration and MET receptor activity (Cooke et al. 2012). As a result, the tumor vasculature of "normal" pericyte coverage could operate as a very essential inhibitor of proliferation.

1.3.10 Lymphatic Endothelial Cells

Tumors produce VEGFC or VEGFD, which causes lymphangiogenesis or lymphatic hyperplasia (Lahdenranta et al. 2009). The TME will experience broad lymphatic artery sprouting, enlargement of accumulating lymphatic vessels, and lymph node lymphangiogenesis if cancerous tumors or macrophages generate significant concentrations of VEGFC or VEGFD, even though tumor cells can access already-existing lymphatic channels. There is gradually emerging that lymphatic endothelial cells impact tumor progression by physiologically regulating the TME and changing the host defense to the tumor, in addition to their critical involvement in the lymphatic vasculature and TME in the dispersion of cancer cells (Padera et al. 2016).

1.4 Diagnosis and Treatment of Cancer

1.4.1 Diagnosis of Cancer

Diagnosing cancer at a late stage leads to ineffective treatment options and increased mortality because of the progression in the near cells/organ uncontrollably. A reduction in mortality can be achieved if the cancer is diagnosed at an early stage

which helps in the surgical removal of the tumor and treatment is possible with milder drug regimens (World Health Organization 2017). Early diagnosis has been reported to increase the survival rate up to 91% on an average of 5 years while up to 26% at late diagnosis (Bray et al. 2018). Currently, methods for early cancer diagnosis include morphological examination of tissues (histology) or cells (cytology) and imaging techniques. Cancer can only be identified using the most popular imaging methods, such as X-rays, MRIs, computed tomography, endoscopy, and ultrasound when there appears a clear-cut tissue alteration (Zhang et al. 2019). Thousands of cancer cells could have proliferated and spread by that point. Additionally, it is impossible to discern between benign and malignant tumors using existing imaging techniques (Panunzio and Sartori 2020). Regardless, nuclear medicine imaging methods are more effective for clinicians to make appropriate decisions and a well-tolerated procedure that can be used to accurately assess the extent of the tumor throughout the clinical history of the disease. Single-photon emission computed tomography (SPECT), positron emission tomography (PET), and hybrid systems are used in these imaging techniques (Atlihan-Gundogdu et al. 2020). Furthermore, early-stage cancer cannot be accurately and independently detected by cytology or histology. Therefore, it is extremely difficult to establish methods for identifying cancer at an early stage, before metastasis (Zhang et al. 2019). Nowadays, several novel diagnosis techniques have been developed which are efficient in the early diagnosis of cancer. These include the use of radiopharmaceuticals, nanoparticle-based cancer diagnosis, T cell-based cancer diagnosis, noninvasive methods, artificial intelligence-based diagnosis, DNA/RNA-based computational diagnosis, and diagnosis through various biomarkers.

1.4.1.1 Radiopharmaceuticals for Cancer Diagnosis

Radiopharmaceuticals contain radionuclides as well as pharmaceutical ingredients. The pharmaceutical component allows radiopharmaceuticals to be delivered to specific organs, tissues, or cells. In order to treat tissue, radionuclides are either employed to scan the tissue or to irradiate the necrosis site. A growing range of radioactive medications is being used in therapeutic settings, enabling in-depth knowledge of the properties of various tumor kinds (Atlihan-Gundogdu et al. 2020). In contrast, radioligand theranostics in oncology leverage molecular imaging, such as neuroimaging, single-photon emission computed tomography, and planar scintigraphy, to diagnose, monitor, and coordinate care (Barca et al. 2021). Biomarkers such as gene or protein expression for a specific disease are targeted through radiopharmaceuticals in molecular imagining and their distribution in the organism is visualized through the emission of radioactive particles. Particles that are emitted by the isotopes are the key factor that defines their imaging and therapeutic applications. Gamma (γ) ray emitters and positron emitters are generally used for diagnosis through SPECT and PET imaging, respectively. Sources of gamma (γ) ray emitters are technetium-99 m (99mTc, T1/2 = 6.0 h), iodine-123 (123I, T1/2 = 13.2 h), and positron emitters are carbon-11 (11C, T1/2 = 20.4 min), fluorine-18 (18F, T1/2 = 109.6 min) and gallium-68 (68Ga, T1/2 = 1.13 h) (Drude et al. 2017). Peptide, antibodies, aptamers, and small molecules are employed for

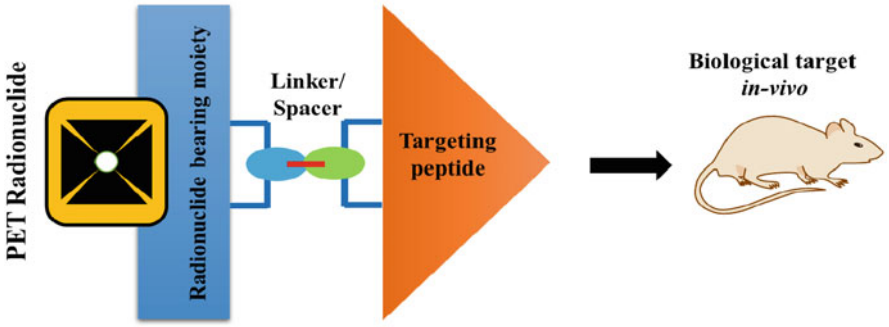

Fig. 1.5 Peptide-based PET radiopharmaceuticals structural components which target the cancer site

diagnosis through radiolabeled biomolecules among which the development of peptide-based radiopharmaceuticals is gaining visualization among the investigators due to their favorable pharmacokinetic properties due to its small size, simple preparation method with various radiolabeling techniques, good resistance to radiolabeling and rapid wash out time with low toxicity. All these properties of peptides offer a better diagnosis of cancer (Ahmadpour and Hosseinimehr 2019). The radiolabeled peptide in Fig. 1.5 consists of a PET radionuclide, a targeting entity, a linker, and a radionuclide carrying moiety.

Nakamoto et al. developed 18F-FP-R01-MG-F2, a novel cysteine-node-peptide PET-based radiopharmaceutical for the diagnosis of pancreatic cancer. The developed radiolabeled peptide selectively binds to human αvβ3 integrin, which is overexpressed in pancreatic cancer (Nakamoto et al. 2021). αvβ6 integrin, which is another excellent biomarker for cancer prognosis, is overexpressed in tumor and tumor endothelial cells in many types of cancer. The Arg-Gly-Asp motif, a radioactive pharmaceutical widely used in positron emission tomography of tumor angiogenesis, has been reported to have a high affinity and selectivity for αvβ3 integrin (Gyuricza et al. 2021). Furthermore, Corlette et al. have developed Ga-68-labeled radiotracer peptides that exhibited enhanced folding capacity with increased biological potency for tumor diagnosis (Table 1.1).

1.4.1.2 Artificial Intelligence in Cancer Diagnosis

In recent years, clinical cancer research has made extensive use of artificial intelligence (AI), particularly machine learning and deep learning, with cancer prediction performance reaching new heights. The use of multifactorial analysis, conventional logistic regression, and Cox analyses to increase diagnosis accuracy has been achieved because of technological advancements in statistics and computer software. It was discovered that these predictions were a lot more accurate than empirical predictions. AI has recently been used by scientists to develop models that employ AI algorithms to predict and detect cancer. These approaches currently play an important role in improving cancer susceptibility, recurrence, and survival prospects

Table 1.1 Different radioactive peptides and their studies on various cell lines

Peptide	Description	Cell line/Receptor	References
^{99m}Tc-HYNIC-(tricine/EDDA)-Lys-FROP peptide	The binding constant to MCF-7 cells was found to be 158 nM and planar gamma imaging showed that the tumor was visible due to uptake in mouse after 15 min of peritoneum injection (p.i.).	MCF-7	Ahmadpour et al. (2018)
^{68}Ga-leuprolide peptide	The peptide, which was radiolabeled with the radionuclides ^{68}Ga and ^{177}Lu, demonstrated nanomolar binding to all three of the examined cell lines, MCF7, T47D, and MDA-MB-231. 45 min after injection, it was discovered that the estrogen receptor-positive MCF7 tumor had accumulated 2.24% × 0.62% ID/g, with good tumor-to-blood and muscle uptake ratios.	MDA-MB-231, MCF-7, T47D	Okarvi and Al-Jammaz (2022)
[^{68}Ga]Ga-DOTA-TOC	High affinity for the somatostatin receptor administered intravenously.	Somatostatin receptor	Hennrich and Benešová (2020)
[^{68}Ga]Ga-ABY-025	Four hours after high peptide content injection, there was no overlap, and absorption in HER2-positive lesions was five times larger than in HER2-negative lesions.	Human endothelial growth factor receptor type 2	Velikyan et al. (2019)
[^{177}Lu]Lu–DOTA–p160	Uptake experiments on the MCF-7 cell line have shown saturable radio-conjugate binding in the nanomolar range.	MCF-7 (HER-2)	Kaur et al. (2021)
^{89}Zr-DFO-heterodimeric peptide	It was discovered that the tumor absorption in U87MG xenograft	Vascular endothelial growth factor receptor	Liu et al. (2022)
68Ga-labeled cyclic RGD peptide	The dissociation constant was found to be 15.28 nM and more than 95% of the radioactivity was internalized and surface-bound to A549 cells.	αvβ3 integrin receptors	Pirooznia et al. (2020)
68Ga-iPSMA-BN	High stability against enzyme, heterodimer, >60% activity, increased cellular uptake, low binding with serum, more bioavailability.	Gastrin-releasing peptide receptor, PC3 cell line, and prostate-specific membrane antigen (PSMA) (GRPr)	Mendoza-Figueroa et al. (2018)

(Huang et al. 2020). Chen and his colleagues developed a low-cost solution for cancer diagnosis and staging using an augmented reality microscope (ARM). The researchers demonstrated the use of ARM to detect metastatic breast cancer and identify prostate cancer with latency compatible with real-time use (Chen et al. 2019). Strom et al. devised a clinically acceptable AI system for prostate cancer detection, localization, and Gleason classification. The digitized core needle biopsies from randomly selected participants and the use of resulting images to train deep neural networks in evaluating prostate biopsies (Ström et al. 2020). On the other hand, Horie et al. developed and validated an Artificial Intelligence Diagnostic System (GRAIDS) for detecting upper gastrointestinal cancers using imaging data from clinical endoscopies. In this study, they collected 8428 sampling images of esophageal cancer from patients and used these to develop deep learning methods using convolutional neural networks (Horie et al. 2019). Another study looked at the potential benefits of combining human and artificial intelligence for grading melanoma. New deep learning techniques were used to train a single convolutional neural network using 11,444 dermatoscopy images and the human–machine group achieved an accuracy of 82.95% (Hekler et al. 2019). Kawakami et al. used machine learning methods based on multiple biomarkers to create an ovarian cancer-specific predictive framework for clinical stage, histology, residual tumor burden, and prognosis. They employed seven supervised machine learning classifiers, including the gradient boosting machine (GBM), neural network, conditional radio frequency (CRF), boost vector machine, random forest (RF), naive Bayes, and elastic network, to obtain diagnostic and prognostic information. They discovered that machine learning techniques outperformed traditional regression-based analyzes in predicting multiple clinical criteria associated with epithelial ovarian cancer (Kawakami et al. 2019).

1.4.1.3 Nanotechnology in Cancer Diagnosis

Potential solutions, such as nanotechnology, are being employed to address the increased systemic toxicity and refractoriness associated with a current cancer diagnosis and treatment technologies to achieve/have better detection and lower the severity of the disease (Jin et al. 2020). Nanotechnology has been investigated as a means to detect extracellular cancer biomarkers and cancer cells, as well as for in vivo imaging, due to its high sensitivity, specificity, and multiplexing capabilities (Zhang et al. 2019). Numerous nanoparticles, nanotubes, liposomes, nano micelles, branched dendrimers, nanocapsules, nanostructured lipid formulations, and other carriers have been used in the development of cancer drugs, showing important pharmacokinetic and pharmacodynamic advantages in the detection and treatment of cancer (Barani et al. 2021). A variety of nanoparticles are now utilized for molecular imaging. Nanoparticles have been employed extensively in cancer detection and surveillance in recent decades. Their benefits, including their tiny size, strong biocompatibility, and high atomic number, have made them popular in cancer research and diagnostics (Shrivastava et al. 2021). Nanoparticles with unique optical, magnetic, or structural features are employed in the diagnosis of cancer, including semiconductors, quantum dots, and iron oxide nanocrystals (Popescu

et al. 2015). They are being used in cancer diagnosis to capture cancer biomarkers such as cancer-associated proteins, circulating tumor DNA, circulating tumor cells, and exosomes (Jia et al. 2017). Rajkumar and prabaharan have formulated multi-functional Core-shell Fe_3O_4@Au nanoparticles conjugated with doxorubicin for cancer theranostic applications. Researchers suggested that the developed formulation could be a viable tumor-targeted drug delivery system that could be used for MR/CT imaging, photothermal treatment, and chemotherapy (Rajkumar and Prabaharan 2019). Based on gold nanoparticles (AuNPs) labeled with biotinylated poly(adenine) ssDNA sequences and streptavidin-horseradish peroxidase for enzymatic signal enhancement, Huang et al. developed a colorimetric method for cancer biomarker detection. They eliminated the complicated and costly thiol-binding process by using AuNP-modified surfaces with ssDNA. In addition, these surface-modified AuNPs can be incorporated into a paper-based, smartphone-reading analytical device that can perform multiple, simultaneous tests with low sample consumption. Applying these innovations, they were able to detect 10 pg/mL of prostate-specific antigen with a test that took 15 min to complete (Huang et al. 2018). Furthermore, in cancer diagnosis, visible spectral imaging is limited by its inability to penetrate objects. In order to overcome this issue, quantum dots emitting fluorescence in the near-infrared spectrum (e.g., 700–1000 nm) have been developed, improving the ability of these dots to detect colorectal cancer, liver cancer, pancreatic cancer, and lymphomas. In a recent study, it was reported that silver-rich Ag_2Te quantum dots (QDs) containing a sulfur source can be used to enable better spatial resolution images in the near-infrared range (Zhang et al. 2020). Nanoshells are another commonly used nanotechnology application. The dielectric cores are typically made of silicon and coated with a thin metal shell (usually gold). A UV-infrared emission/absorption array in the nanoshell enables flexible optical tuning through the conversion of plasma-mediated electrical energy into light energy. Despite their large size, nanoshells are desired for their lack of heavy metal toxicity and their ability to produce high-quality imaging (Jin et al. 2020). An SERS immunosensor based on an Au@SiO_2 array substrate and Au-Ag nanoshell probes was developed by Ran et al. for ultrasensitive detection of surviving and osteopontin in cervical cancer serum. Based on their experimental results, the constructed SERS immune-sensor demonstrated satisfactory selectivity and reproducibility while having detection limits of 0.908 pg/mL and 0.813 pg/mL for surviving and OPN in humans serum, respectively (Ran et al. 2022).

1.4.2 Treatment of Cancer

Cancer remains a leading cause of death worldwide despite significant advances in treatment (Wei et al. 2021). The treatment of cancer may include surgery, radiotherapy, and systemic therapy. When the disease is in an early stage, surgical treatment alone is often enough to cure low-risk patients. However, in most cases, a combination of treatments is necessary. When the disease has spread to distant sites, the primary therapeutic modality is systemic therapy since it can be delivered to

disseminated cancer sites through the bloodstream. Systemic therapies include hormonal therapy, targeted therapy, immune therapy, and chemotherapy (Dickens and Ahmed 2018). Chemotherapy uses toxic drugs to kill cancer cells. There have been several new chemotherapeutic drugs discovered and synthesized since World War II (Wei et al. 2021). Chemotherapeutic agents are divided into four categories according to their mechanism of action at the molecular level. Alkylating agents, antibiotics, nucleotide reductase inhibitors, related antimetabolites, and anti-tumor plant medications make up the four groups (Fig. 1.6) (Sun et al. 2021).

Chemotherapeutic drugs frequently interfere with the cell cycle in order to achieve their effects. In cancerous cells, the cell division process is unlike the normal cell division process. Due to the mutation of proto-oncogenes and tumor suppressor genes, the cell acquires the ability to inhibit the growth inhibitory signals and ultimately leading to cell immortality. The cell cycle is composed of four phases that are M phase (Mitosis), G-1 phase (Gap), S-phase (Synthesis), and G-2 phase (Gap). Synthesis of DNA takes place in S-phase and the separation of chromosomes occurs in the M-phase (Sun et al. 2021). Chemotherapeutic drugs work either by directly inhibiting DNA synthesis or by targeting the key elements of the cell cycle. Radiation treatment and radiation medications, bifunctional alkylating chemicals, topoisomerase inhibitors, replication inhibitors, and DNA damage can all prevent

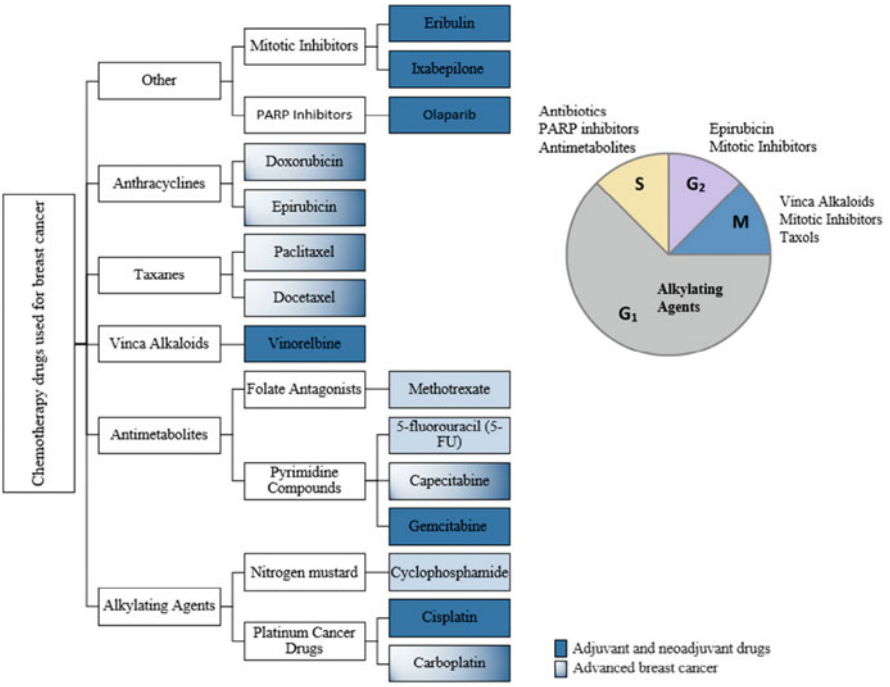

Fig. 1.6 Classification of chemotherapy drugs according to the cell cycle phase they are active in the treatment of breast cancer (Pralea et al. 2020)

normal DNA synthesis by destroying DNA chains, preventing base pair binding, or interfering with nucleotide anabolic metabolism (Lama-Sherpa and Shevde 2020). Additionally, reactive oxygen species can harm DNA (Aggarwal et al. 2019). Doxorubicin, carmofur, mitomycin C, gemcitabine, mercaptopurine, camptothecin, paclitaxel, mitoxantrone, vinblastine, cisplatin, procarbazine, and quinone medicines are chemotherapy medications that can cause oxidative stress (Yokoyama et al. 2017). Some essential components, such as cyclins and cyclin-dependent kinase (CDK), which control cell cycle checkpoints and signaling pathways, carry out the regulation of the cell cycle. Cyclins are regulatory proteins that bind with their corresponding protein kinases to promote cell division. At various phases, retinoblastoma (Rb), CDKs, and CDK inhibitors regulate the amount of cyclin in mammalian cells. Cyclin-deficient cells cannot pass the G1/S border (Jirawatnotai et al. 2020). Cell cycles with different cyclins and CDKs with their checkpoints are depicted in Fig. 1.7.

Examples of first-generation CDKs inhibitors having anticancer properties are flavopiridol, lomustine, roscovitine, and kenpaullone whereas second-generation CDKs inhibitors are roniciclib, dinaciclib, voruciclib, and riviciclib (Julve et al. 2021). Nonetheless, these drugs also affect the normal cells and their division

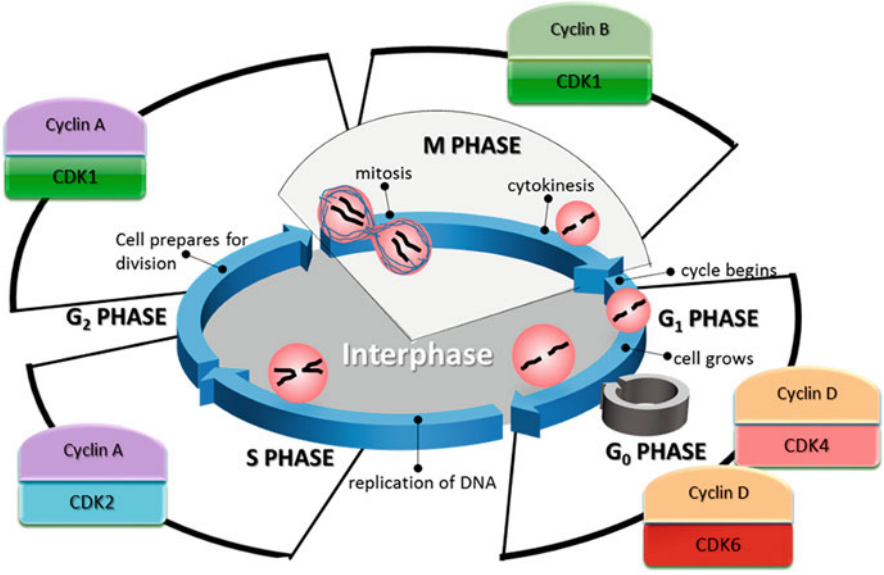

Fig. 1.7 The cell cycle phases and their associated cyclin-dependent kinases (CDK)/cyclin complexes. In the G1 phase of the cell cycle, the synthesis of cyclin D is increased. This cyclin partners with CDK4/6 to promote cell cycle entry, and its progression through G1, as well as the G1/S transition. During the S phase, CDK2 in complex with cyclin A controls the phosphorylation of targets involved in DNA replication. Cyclin A is found highly expressed in this phase and until the last stages of G2. In the G2 phase, the primary regulator of the cell cycle is CDK1 (García-Reyes et al. 2018)

process, mainly the cells that are in continuous division phases such as cells of bone marrow and mucous membrane hence targeting such cancerous cells is very much important. Therefore, tumor-specific targeting is very much essential in order to treat cancerous cells. Tumor-specific targeting remains a big challenge for researchers and scientists nowadays. Extensive research is going on for developing a targeted drug delivery system of chemotherapeutic agents. Targeting of tumor cells can be possible by mechanisms, i.e., passively targeting and active targeting. In passive targeting, the enhanced penetration and retention of the drug delivery system (DDS) takes place through the tumor blood vesicle and makes a complex niche with the tumor cells. This prevents the vesicle system to leak out from the tumor microenvironment and its clearance. Whereas active targeting is implicated after the passive accumulation of DDS in the tumor. It entails smearing tumor-specific ligands, including aptamers, antibodies, and receptors overexpressed by tumor cells, on the surface of drug carriers (Sun and Zhong 2020). It is possible to target cancer cells by targeting different receptors that regulate different pathways. A variety of drugs that inhibit pathways through the receptors are listed in Table 1.2.

Furthermore, cancer drug delivery has greatly benefited from nanotechnology. Tunable prodrugs, polymeric micelles, inorganic nanoparticles, nanotubes, nanorods, dendrites, lipid-based drug delivery systems, and carrier-based drug delivery systems are now available as nanotechnology-based drug delivery systems for cancer cell treatment (Rahim et al. 2021). Recently developed nanoformulations as cancer treatment strategies are included in Table 1.3.

Alternative anticancer treatment methods are urgently required due to specific action, side effects, and the emergence of resistance. A promising new approach involves the use of naturally derived microbial toxins against cancer cells. Aflatoxin, diphtheria toxin, vibrio cholera toxin, patulin, cryptophycin-55, and chlorella are only a few examples of significant microbial toxins that come from microbes, molds, and microalgae. Inhibiting protein synthesis, reducing cell growth, regulating cell cycle and many other cellular processes are among the different bio targets of these agents (Sharma et al. 2021). Toxins produced by bacteria act primarily by targeting protein molecules and by modifying immune responses through DNA (Weerakkody and Witharana 2019). Several bacterial toxins isolated from different sources are known to have anticancer activity. They are colibactin, cytolethal distending toxin, diphtheria toxin, *clostridium perfringens*, *Pseudomonas aeruginosa* exotoxin A, botulinum toxin, alpha-toxin, cytolysin A, cholera toxin, adenylate cyclase toxin, and listeriolysin O (Zahaf and Schmidt 2017). It seems that fungal toxins alter the cell cycle and have a higher potential to damage DNA. Examples of fungal toxins are aflatoxins, ochratoxin A, trichothecenes, nivalenol, deoxynivalenol, citrinin, and patulin (Awuchi et al. 2022). Contrarily, algal toxins include chlorella, astaxanthin, fucoidan, *grateloupia longifolia* polysaccharide, and cryptophycin-55 (Sharma et al. 2021).

Table 1.2 Role of various anticancer drugs which inhibit the growth of cancer by targeting different receptors

Receptors	Inhibitors	References
Fibroblast Growth Factor Receptors (FGFRs)	Erdafitinib (JNJ-42756493)	Roubal et al. (2020)
	Pemigatinib (INCB054828)	Weaver and Bossaer (2021)
	AZD4547	Chae et al. (2020)
	Rogaratinib (BAY1163877)	Grünewald et al. (2019)
	Lucitanib (E-3810, CO-3810)	Liang et al. (2021)
	Debio1347	Cleary et al. (2020)
	Futibatinib (TAS-120)	Sootome et al. (2020)
	Derazantinib (ARQ087)	Mcsheehy et al. (2019)
	Infigratinib (BGJ398)	Botrus et al. (2021)
	Zotatifin (eFT226)	Gerson-Gurwitz et al. (2020)
	Anlotinib	Li (2021)
	Lenvatinib (E-7080)	Hoshi et al. (2019)
Epidermal Growth Factor Receptor (EGFR) Tyrosine kinase	Gefitinib (ZD1839)	El Guerrab et al. (2020)
	Erlotinib (CP358774)	Saito et al. (2019)
	Lapatinib (GW572016)	Cooper et al. (2021)
	Icotinib (BPI2009)	He et al. (2021)
	Afatinib (BIBW2992)	Masood et al. (2019)
	Neratinib (HKI272)	Aljakouch et al. (2018)
	Dacomitinib (PF-299,804)	Nagano et al. (2019)
	WZ4002	Pawara et al. (2022)
	Rociletinib (CO1686)	Yang et al. (2021)
	Osimertinib (AZD9291)	Leonetti et al. (2019)
	Olmutinib (HM61713)	Noh et al. (2019)
	EAI001, EAI045	Zhao et al. (2018)
Fibroblast growth factor (FGF), Vascular Endothelial Growth Factor (VEGF), platelet-derived growth factor receptors (PDGF)	Regorafenib	Liu et al. (2020)
	Nintedanib	Zhou et al. (2020)
	Lenvatinib	Al-Salama et al. (2019)
	Anlotinib	Luan et al. (2021)
VEGF, PDGF	Sorafenib	Wang et al. (2018)
VEGF	Fruquintinib	Xu et al. (2022)
VEGF, Stem Cell Factor (SCF)	Apatinib	Shao et al. (2020)
VEGF, PDGF, SCF	Sunitinib	Yuan et al. (2018)
	Pazopanib	Longhi et al. (2019)
	Axitinib	Siedlecki et al. (2018)
The Hepatocyte Growth Factor (HGF), VEGF, PDGF, SCF	Cabozantinib	Jia et al. (2022)

Table 1.3 Some of the recently developed formulations for the effective treatment of cancer

Delivery system	Drug molecule	Target site	Outcomes	References
Nanoparticles	Cabazitaxel-loaded human serum albumin	Folate receptor	HeLa cells and HeLa xenograft tumors responded favorably to the produced formulation's enhancement of FR-mediated endocytosis. The amount of formulation that accumulated at the tumor location was also noticeably increased.	Sun et al. (2019)
PLGA-nanoparticles	Methotrexate and curcumin	Folate	Nanoparticles showed a significant increase in cytotoxicity when compared to free drugs, and in vivo tests showed that co-delivery inhibited breast cancer progression synergistically.	Vakilinezhad et al. (2019)
Gold nanoparticles	Betulin	–	According to the in vitro findings, betulin-coated gold nanoparticles exhibited a dose-dependent cytotoxic impact and caused apoptosis in all of the examined cell types.	Mioc et al. (2018)
Solid-lipid nanoparticles	Folic acid conjugated trans-resveratrol-ferulic acid	Folate receptor	Using the developed formulation, the drugs were delivered effectively and apoptosis was induced. In addition, it regulates the expression of cyclin proteins and cell cycle arrest.	Kumar et al. (2020)
RGD-conjugated chitosan nanoparticles	Raloxifene	αvβ3 integrin	Studies in hematology and blood biochemistry have shown that the produced nanoparticles may be able to recognize malignancies and	Yadav et al. (2020)

(continued)

Table 1.3 (continued)

Delivery system	Drug molecule	Target site	Outcomes	References
			prevent tumor development without having detrimental effects on healthy tissue.	
Lipid-based nanoparticles	Paclitaxel	EGFR	While an in vivo investigation using a mouse xenograft model for ovarian cancer revealed that the formulation has decreased the tumor burden by up to 50% in all tissue, and in vitro examination using HEY ovarian cancer cell lines revealed that the formulation had increased cytotoxicity.	Zhai et al. (2018)
Superparamagnetic iron oxide nanoparticles	Curcumin	Sonic hedgehog (SHH) pathway and CXCR4/CXCL12 signaling	Due to the overexpression of human nucleoside transporter genes (DCK, hCNT) and inhibiting ribonucleotide reductase subunits (RRM1/RRM2), the proposed drug delivery system successfully delivered curcumin to pancreatic tumor cells with improved GEM uptake. Additional examination of tumor tissues revealed that the formulation both causes a change in cell stiffness and suppresses the tumor stroma.	Khan et al. (2019)
Folate-modified PLGA nanoparticles	Paclitaxel	Folate	For nanoparticles modified with folate, cellular uptake was 3.6 times greater than for their unmodified counterparts in SKOV-3 cells.	Luiz et al. (2019)

(continued)

Table 1.3 (continued)

Delivery system	Drug molecule	Target site	Outcomes	References
Albumin nanoparticles	Paclitaxel and difluorinated curcumin	Folate	The combination of both the folate conjugated formulation showed a synergistic anticancer efficacy and showed an augmented uptake as well as induction of apoptosis.	Gawde et al. (2018)
Liposomes	Echinomycin	HIF-1α	Compared to traditional echinomycin, liposome-formulated echinomycin inhibits primary tumor growth significantly more and eliminated established triple-negative breast cancer metastases.	Bailey et al. (2020)
Liposomal modified with bombesin peptide analog	Doxorubicin	GRP	In vivo study showed that the mice treated with modified pegylated liposomal Dox have reduced tumor growth as well as improved efficacy than the non-modified one.	Accardo et al. (2019)
GE11 peptide-modified reversibly cross-linked polymersome	Doxorubicin	EGFR	In vitro studies showed that the formulation showed higher uptake in SKOV3 cell lines whereas in vivo studies showed that the biodistribution of the formulation is 2.5-fold higher in the tumor cells. Furthermore, a single dose of 60 mg/kg of formulation showed effective treatment with lower toxicity in mice.	Zou et al. (2018)
Nano-liposomes	Talazoparib	PARP	It has been demonstrated that Talazoparib nano-formulated improves therapeutic effectiveness while	Zhang et al. (2019)

(continued)

Table 1.3 (continued)

Delivery system	Drug molecule	Target site	Outcomes	References
			lowering off-target toxicities in BRCA-deficient animals, and this may also be true for people with BRCA-deficient breast cancer.	
Liposomes	Gemcitabine and Clofazimine	DNA synthesis	In comparison to other liposome treatments, the GEM/CLF co-loaded liposome treatments displayed greater percentages of apoptotic cells, caspase-3 activity, and mitochondrial membrane depolarized cells. In this work, CLF's cytotoxicity toward bone cancer cells as well as its synergistic interactions with GEM on osteosarcoma both described for the first time.	Caliskan et al. (2019)
Liposomes	Itraconazole	Hedgehog (Hh) pathway	Liposomes with the optimal size for penetration of the BBB that contain ITZ were developed.	Pace et al. (2020)
Liposomes	Paclitaxel and vinorelbine	Folate	As a result of biodistribution studies conducted on C57BL/6 mice bearing NSCLC tumors, radiolabeled, actively folate targeted liposomal formulations with co-drugs encapsulated were found to be more effective at targeting tumor cells than nontargeted formulations. Comparing folate-targeted, co-drug	Karpuz et al. (2021)

(continued)

Table 1.3 (continued)

Delivery system	Drug molecule	Target site	Outcomes	References
			encapsulating liposomal formulation to free drugs, the folate-targeted formulation provided more effective treatment.	
Aptamer-labeled liposomal nanoparticles	Doxorubicin	Her-2	Studies on cytotoxicity have shown that the DOX-loaded liposome formulation is, in comparison to free DOX, more effective against the MCF-7 and SKBR-3 cell lines. As opposed to non-aptameric liposomes, Her-2+ MCF-7 and SKBR-3 breast cancer cells showed more than 60% absorption of aptameric liposomes, according to a research on cellular uptake.	Chowdhury et al. (2020)
Liposomes	Doxorubicin and ceramide	PI3K/Akt pathway	When compared to DOX liposomes containing no ceramide or DOX solution, C8-ceramide-based liposome formulations showed significantly higher cytotoxicity in B16BL6 melanoma cell lines.	Chen et al. (2019)
Mesoporous silica nanoparticles	Gadolinium	Folate	Both photodynamic therapy and photothermal therapy effects could be achieved with the developed formulation, as well as near-infrared fluorescence imaging and photoacoustic imaging are also possible.	Sun et al. (2018)

(continued)

Table 1.3 (continued)

Delivery system	Drug molecule	Target site	Outcomes	References
Hyaluronic acid conjugated dendrimer-based nanoparticles	Doxorubicin and siMDR-1	Downregulating the membrane-bound P-gp and Her-2 receptor targeting	The cationic charge of the HA-modified MDMs has reduced their toxicity against cancer-specific cells and increased their ability to kill cancer cells on the CD44+ cell line.	Zhang et al. (2020)
Poly-amidoamine dendrimer nanocapsules	Trastuzumab (monoclonal antibody) and Neratinib	Her-2	In vitro research has shown that TZ-targeted dendrimers combined with neratinib had a higher antiproliferative effect and selectivity against SKBR-3 cells.	Aleanizy et al. (2020)
N-acetyl-D-glucosamine-labeled PAMAM dendrimers	Camptothecin	Glucose transporters and lectin receptors	Developed formulations have shown dose and time-dependent anticancer activity. NAG-Dend-CPT has shown to increase the reactive oxygen species generation with a higher apoptosis rate and greater inhibition activity on the A549 cell line in comparison to Dend-CPT.	Pooja et al. (2020)
Polyamidoamine dendrimers	Cisplatin and doxorubicin	Crosslinking with purine bases of DNA and Inhibiting topoisomerase 2.	In vitro tests of HA@PAMAM-Pt-Dox revealed a time-dependent entrance route. Research on the survival of the breast cancer cells MCF-7 and MDA-MB-231 revealed that HA@PAMAM-Pt-Dox displayed a stronger anticancer activity at a relatively low concentration.	Guo et al. (2019)
PAMAM dendrimers	Paclitaxel	Biotin	By flow cytometry and confocal microscopy study, it was concluded that the	Rompicharla et al. (2019)

(continued)

Table 1.3 (continued)

Delivery system	Drug molecule	Target site	Outcomes	References
			biotin conjugated formulation penetrated more in monolayers and spheroids.	
Niosomes	Withaferin–A	CD31 marker cells	By SRB assay it was found that the WA-niosome showed a 3 times increased anticancer activity than the pure one.	Shah et al. (2020)
Folic acid-functionalized niosomal nanoparticles	Curcumin and letrozole	Folate	In comparison to letrozole and curcumin alone, letrozole/curcumin-loaded niosomes increase the rate of apoptosis in MCF-7 and MDA-MB-231 cells.	Akbarzadeh et al. (2020)

1.5 Conclusion and Future Aspects

Present drug delivery modules based on different nanotechnological modifications involved in the targeting of cancer, aim to improve therapeutic outcomes while reducing the toxic effects. The delivery system could be modified by different synthetic and biological molecules, i.e., cell-penetrating peptide (CAR nonapeptide, TAT, iRGD, etc.), metallic modifications (gold, iron, silver, platinum, zirconium, nickel, etc.). Additionally, research has shown that the use of probiotics and prebiotics, both singly and in combination (synbiotics), is beneficial in the management and protection of a variety of serious ailments, including cancer, HIV infection, digestive problems, and many others. For a complete knowledge of the structure and function of the microbiome relating to probiotics and prebiotics, modern approaches based on molecular biology, system biology, genetic engineering nanotechnology, multi-omics, and immunology must be used. For the prevention of cancer, scientists and researchers from academic institutions, doctors, and companies should collaborate and work together in this direction through translational research projects that aim to immediately link ideas from the lab to the producers, the public, and the medical community.

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Effect of Anticancer Treatment Approaches on Gut Microbiota

2

Ruchika Sharma and Anoop Kumar

Highlights

- Resistance to anticancer treatments might be linked with altered gut microbiota.
- Tumor-associated bacteria may be used as a diagnostic or prognostic marker for cancer as per the preclinical and clinical evidence.
- Antibiotics, synbiotics, probiotics, and prebiotics that alter the microbiota may enhance the anticancer effects of anticancer drugs.

2.1 Introduction

The gut microbiota (GM) is not affecting only gastrointestinal (GI) system but also involved in maintaining the health of other systems like nervous system (Rowland et al. 2018). Because of their proximity to the immunological milieu within the gastrointestinal tract, microbes in the human gut have a significant influence on health and immune function, which has been dubbed "the last unexplored human organ" (Wu and Wu 2012). These microorganisms have evolved with humans characterized by long-term processes. Number of bacteria in human body is more than human cells (Sender et al. 2016). The human intestine contains approximately 3.8×10^{13} bacteria weighing nearly 1.8 kg, commonly known as gut microbiota, and normal human GM is divided into two primary phyla, Bacteroidetes and Firmicutes (Sender et al. 2016; Thursby and Juge 2017; Stojanov et al. 2020). Pharmacomicrobiomics emphasizes on the interaction between the gut microbiota and drug response (Ting et al. 2022). The gut microbiota is now widely acknowledged for

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sustaining physiology and health of the host by performing number of tasks. To accomplish these goals, the microbiota forms a eubiosis (equilibrium with the host) relationship. However, changes in the makeup and function of the gut microbiome (dysbiosis) play a role in the development of a variety of disorders, including obesity, diabetes, neurodegenerative diseases, and cancer (Durack and Lynch 2019). Emerging studies have indicated that the gut bacteria can modulate the efficacy or toxicity of chemo- and immunotherapeutic medicines (Pouncey et al. 2018; Lee et al. 2021). Therefore, microflora show an important role in pathogenesis of cancer as well as in efficacy and safety of anticancer drugs. The microbes are also involved in metabolism of drugs as well as modulate immune responses and inflammation (Durack and Lynch 2019; Panebianco et al. 2018; Bhatt et al. 2017).

This chapter starts with relationship between microbiome and cancer, cancers caused by microbes, microbiota changes reported in cancer. The role of gut microbiota in treatment of cancer with anticancer drugs and immunotherapy are also discussed. Finally, chapter briefly described the future perspective of effect of anticancer treatment approaches on gut microbiota.

2.2 Historical Relationships Between the Microbiome and Cancer

Bacteria, which are predominantly found in the primary and secondary phyla, i.e. bacteroidetes, proteobacteria, firmicutes, and actinobacteria, play an important role in the microbiota (Sender et al. 2016; Hillman et al. 2017). The GM consists trillion of microorganisms and produce thousands of metabolites which are interacting with each other and maintaining homeostasis (Thursby and Juge 2017; Stojanov et al. 2020; Rinninella et al. 2019). There are also a number of microbes which results in the development and progression of cancers (Whisner and Athena 2019). Cancer has been linked to a variety of microbial communities and some of cancers caused by microbes are summarized in Table 2.1.

Microbial activity was responsible for 17.8% of all malignancies in 2002 (Hullar et al. 2014). *Helicobacter pylori* and stomach cancer are an early causative link between a certain bacterial species and human cancer (Plottel and Blaser 2011; Choudhry 2021; Flavell and Murray 2000; Bansal et al. 2016). Warren discovered *H. pylori* and later determined that it was linked to ulcers. The Correa pathway has been developed to track the progression of an *H. pylori* infection to eventual carcinogenesis (Ahmed 2005). *H. pylori*-induced chronic inflammation can lead to atrophic gastritis and dysplasia. Although the findings are still controversial, later after examination, it has been found that the *H. pylori* appears to have a protective effect against esophageal adenocarcinomas, which is surprising (Ahmed 2005; Kunovsky et al. 2021). However, there is a clear connection of *H. pylori* in some of cancers like esophageal adenocarcinoma which are developed after Barrett's esophagus disorder (Ahmed 2005; Naini et al. 2016). The infection with *H. pylori* is inversely associated with Barrett's esophagus. This might be due to lowering the local pH in the stomach's subregions by *H. pylori*, hence lessening the severity of

Table 2.1 List of cancers caused by microbes (identified)

Sr. No	Cancer (type)	Microbe	References
1	Esophageal Gastric adenocarcinoma Gastric lymphoma	*Helicobacter pylori*	Pouncey et al. (2018), Lee et al. (2021), Bhatt et al. (2017), Plottel and Blaser (2011), Choudhry (2021), Flavell and Murray (2000), Bansal et al. (2016)
2	Oropharyngeal carcinoma Anogenital carcinoma	Human Papilloma Virus (HPV)	
3	Lymphoma	Hb-C Virus	
4	Hepatocellular carcinoma	HB-B Virus	
5	Lymphomas Nasopharyngeal carcinoma	Human Immunodeficiency Virus (HIV)	
6	Kaposi sarcoma	Human Herpes Virus 8	
7	Nasopharyngeal carcinoma Burkitt's lymphomas	Epstein–Barr Virus	

GERD symptoms. As a result, microbe can have both tumor-suppressing and tumor-causing qualities; therefore, more research is required to understand these types of phenotypic changes due to interactions of host microbiome (Rubenstein et al. 2014).

Another example is the Epstein–Barr virus (EBV) has been associated to cancer, particularly Burkitt's lymphomas. Although EBV infection may not cause cancer on its own, it may play a role in carcinogenesis when paired with genetic and environmental factors (Bakkalci et al. 2020). There was early conjecture in the case of breast cancer that a mammary tumor virus might play a role in the disease's development in humans. It's well known in mice, however, in human, no comparable virus has been identified. The microbiota changes reported in human cancer types are compiled in Table 2.2.

2.3 Human Tumor Microbiota Interactions

Cancer aetiology has also been linked to specific gut microorganisms, notably gastrointestinal malignancies (Bultman 2014; Abreu and Peek Jr. 2014). There are number of examples such as Fusobacterium nucleatum antigen adhesin A (FadA) promotes the colorectal cancer (CRC), PKS-positive Escherichia coli enhances colorectal tumorigenesis, and *Bacteroides fragilis* toxin induces DNA damage (Gopalakrishnan et al. 2018; Li et al. 2021; Rubinstein et al. 2013; Clay et al. 2022). While some bacteria have direct impacts on tumorigenesis, others induce

Table 2.2 Variations of microbiota in different types of cancer

S No	Cancer type	Microbiota variations		References
		Increases	Decreases	
1	Mouth carcinoma	*Capnocytophaga ochracea*, *Capnocytophaga gingivalis*, *Streptococcus mitis*, *Eubacterium sabureum*, *Leptotrichia buccalis*	*Clostridium* spp.	Mager et al. (2005), Gong et al. (2013)
2	Colorectal	*Streptococcus* spp., *Escherichia coli*, *Bacteroides*, *Staphylococcus bovis*, *Fusobacterium nucleatum*, *Clostridium* spp.	*Lactobacillus*, *Microbacterium*, *Anoxybacillus*, *Akkermansia muciniphila*	Yang and Ji (2019), Khan et al. (2012)
3	Gall bladder	*Salmonella paratyphi*, *Salmonella typhi*	*Lactobacillus* spp.	Sharma et al. (2007)
4	Esophageal and Barrett's esophagus	*Treponema denticola*, *Campylobacter concisus*, *C. rectus*, *S. mitis*	*Helicobacter pylori*	Narikiyo et al. (2004), Macfarlane et al. (2007)

inflammation or decrease immunosurveillance, allowing cancer to develop in an indirect manner. The "immune-oncology-microbiome axis" refers to these microbial immunomodulatory activities (Jain et al. 2021).

The microbiome influences cancer therapy responses in addition to disease. Despite significant advancements in cancer therapy, obstacles such as acquired resistance, side effects, and a wide range of treatment results still exist. The more detailed studies are required to understand the connection of microbiota with diseases, identification of specific taxa as well as to locate potential biomarkers or therapeutic targets, a first-pass characterization (Turnbaugh et al. 2008; Weinstock 2012; Vivarelli et al. 2019; Morgan and Huttenhower 2012; Goodrich et al. 2014; Benson 2016). The most common method for studying these topics are cross-sectional studies in which microbes or group of microbes are identified which are responsible for diseases progression (Goodrich et al. 2014; Benson 2016).

The vast majority of these studies have concentrated on cancers that arise in tissues with a microbial ecology. The exploration of tissue locations generally considered not to sustain resident microbial populations (Table 2.3) (Quail and Joyce 2013).

A unique hypoxic environment flourish bacteria that caused tumors. In a mouse model, Malmgren and Flanigan proved in 1955 that *Clostridium tetani* development is encouraged in the tumor microenvironment (Duong et al. 2019). Because of inadequate vascularization caused by tumor-stimulated angiogenesis, tumors might acquire hypoxic conditions as a result of the expansion of oxygen supply (Muz et al. 2015). Because of the hypoxic and necrotic environment, anaerobic bacteria can proliferate selectively, which is a key feature of the tumor microbiome (Fig. 2.1).

Table 2.3 List of few studies showing the elevation of tumor growth by bacterial microbiome using mouse models

Sr No	Type of cancer	Model	Exposure/treatment	Outcome	References
1	Gastric	Mice (Germ free)	*Helicobacter pylori*	Tumors in mice	Pouncey et al. (2018), Lee et al. (2021), Whisner and Athena (2019), Plottel and Blaser (2011), Choudhry (2021), Schreiber et al. (1972), Lofgren et al. (2011), Lee et al. (2008), Dapito et al. (2012), Yoshimoto et al. (2013), Yu et al. (2010), Li et al. (2012), Klimesova et al. (2013), Islami and Kamangar (2008), Ferreri et al. (2012), Senff et al. (2008), Ammer-Herrmenau et al. (2020), Zhan et al. (2016), Hu et al. (2010), Thomas and Jobin (2020)
		Mice (Antibiotic treated)	*Helicobacter pylori*	Tumors in mice	
2	Liver	Mice (Germ free)	*N*-nitrosodiethylamine and CCl_4	Tumors in mice	
		3,20-dimethyl-4-aminobiphenol-treated mice (Germ free) on a high fat diet	Antibiotic cocktail	Tumors in mice	
		Mice (Germ free) treated with 3,20-dimethyl-4-aminobiphenol	Vancomycin	Tumors in mice	
		Rat (Germ free) treated *N*-nitrosodiethylamine	Neomycin	Less tumors	
3	Lung	Rat (Germ free)	Nitrosoheptamethyleneimine	Less tumors in rats (male)	
4	Colorectal	Mice (Germ free)	Mixture of antibiotics	Tumors in mice	

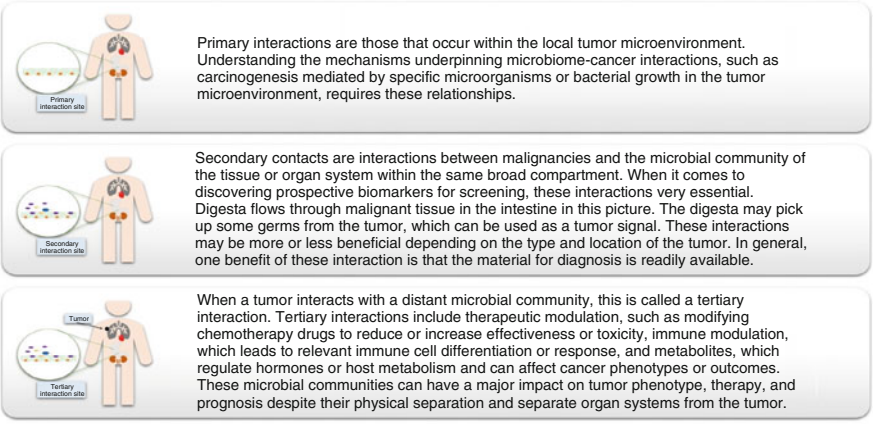

Fig. 2.1 Interactions between the tumor and microbial populations at different levels

2.4 Association Among the Gut Microbiota and Anticancer Treatments

The gut bacteria and anticancer treatments, such as chemotherapy and immunotherapy, have a close association, according to growing data.

2.4.1 Microbiota and Chemotherapy (Efficacy and Toxicity)

Chemotherapy, a systemic cancer treatment incorporating cytotoxic medicines, has greatly improved cancer patients' overall survival rates. Various classes of drugs such as alkylating agents, mitotic inhibitors, and selected antibiotics are being used in the treatment of cancer. Their anticancer effects are based on affecting DNA integrity, as well as DNA repair and synthesis enzymes (Bracci et al. 2014; Falzone et al. 2018). However, the most significant disadvantage of chemotherapy is the occurrence of various types of adverse events (AEs) (Oh et al. 2021; Knoerl et al. 2018). CTX also suppresses immunological responses, which increases the risk of infection and, as a result, morbidity and mortality (Oh et al. 2021).

Various studies in literature have indicated the association of gut microbiome in the pathogenesis of cancers such as breast, colorectal (CRC), ovarian, and prostate (PCa) cancers. Recent studies have also suggested to target microbiota to improve the efficacy and safety of chemotherapy (Sawamura et al. 2022).

Infections with *Mycoplasma* species, particularly *Mycoplasma hyorhinis* have been reported in a variety of malignancies (Yang et al. 2010; Nascimento Araujo et al. 2021). These microorganisms are known to produce nucleoside analog-catabolizing enzymes, which may reduce medication efficacy (Nascimento Araujo et al. 2021; Bullman et al. 2017). The gemcitabine resistance was observed in

animals injected subcutaneously with *M. hyorhinis*-infected colon cancer cells (Rinninella et al. 2019). The resistance of gemcitabine is well known in bacteria due to production of cytidine deaminase (Geller et al. 2017). It has also been observed that the co-administration of ciprofloxacin helps in reversal of gemcitabine resistance in a colon cancer mouse model (Geller et al. 2017; Klemm and Joyce 2015). Similarly, the in vitro study conducted by Lehouritis et al. have shown the decrease in the activities of some drugs and increase in the efficacy of others when used in combinations with bacteria. The influence of chemotherapy on efficacy and safety of anticancer drugs are summarized in Table 2.4.

The platinum compounds are one of the well-known compounds used in the treatment of cancers. The role of microbiota in the efficacy and safety of platinum compounds are also studied (Ting et al. 2022; Gopalakrishnan et al. 2018) and found that the platinum compounds are also able to stimulate immune responses in addition to their classical mechanism of action, i.e., blockage of DNA replication and stimulation of ROS productions. Iida and colleagues have reported the lower tumor regression and survival of subcutaneous EL4 lymphoma mice after given a combination of antibiotics (Iida et al. 2013). The similar results were observed in antibiotic-treated mice with EL4 lymphoma receiving cisplatin with subcutaneous colon cancer and germ-free mice (Liou and Storz 2010; Makovec 2019).

A decrease in microbiota-dependent ROS generation was found as the cause of therapeutic failure. The study conducted by Gui et al. have reported the increase in the size of tumor and lower survival rate in cisplatin combination with antibiotics group as compared to cisplatin alone group in animal model of lung cancer (Gui et al. 2016). However, another study has reported good response of cisplatin in combination with *Lactobacillus* bacteria. The observed effects were linked to regulation of VEGFA, BAX, and CDKN1B gene expression in the tumor, as well as bacterial augmentation of T cell immunity. The therapeutic usage of cyclophosphamide (CTX) as an anticancer agent also depends upon the induction of anticancer immunity. The translocation of group of Gram-positive bacteria into the mesenteric lymph nodes and spleen is occurred which are important in the development of a Th1 and Th17 immune response after CTX therapy in tumor-bearing mice. The animals treated with antibiotics have found resistant to CTX therapy (Anfossi and Calin 2020). In addition, researchers discovered a link between alterations in fecal microbiota and drug-induced gastrointestinal toxicity in rats given irinotecan. Microbial diversity was found to be significantly reduced and linked with intestinal inflammation. The role of microbiota in the efficacy and safety of chemotherapy is presented in Fig. 2.2.

2.4.2 Microbiota and Immunotherapy (Efficacy and Toxicity)

Chemotherapy resistance and recurrence are common issues. Immunotherapy's rapid progress during the last decade has changed treatment guidelines in oncology. Immunotherapy was developed as a novel and alternative approach in clinical oncology in which immune cells fights against the cancer (Szczyrek et al. 2021;

Table 2.4 Influence of chemotherapy on intestinal microbiota profiles (efficacy and toxicity)

S No	Chemotherapeutic treatment	Microbes	Mechanism	Outcome	References
1	5-fluorouracil	*Escherichia coli*	The efficacy of 5-FU is increased through imbalance of bacterial ribonucleotide metabolism and deoxynucleotide	Increase in gram-negative anaerobes	Ting et al. (2022), Stringer et al. (2008), Dabek et al. (2008), McIntosh et al. (2012), Bhatt et al. (2020), Scott et al. (2017), García-González et al. (2017), Viaud et al. (2013), Iida et al. (2013), Bachem et al. (2019), He et al. (2021), Ventola (2017)
		Fusobacterium nucleatum	TLR4/MYD88-dependent pathways activation	*Clostridium* spp., *Staphylococcus* spp., and *Escherichia coli (increases)* *Lactobacillus* spp. and *Bacteroides* spp. (decreases)	
2	Irinotecan (CPT-11)	*Clostridium* clusters XIVa and IV	Reactivation of SN-38G to SN-38 in the gut by bacterial β-glucuronidase which results in significant toxicity	Increased *Clostridium* cluster XI (including *Peptoclostridium difficile*) and *Enterobacteriaceae*	
3	Cytarabine, daunorubicin, and etoposide; amsacrine, cytarabine at high doses	Unspecified	Unspecified	Decrease in Bacteroides spp., *Clostridium* cluster XIVa, *Faecalibacterium prausnitzii*, and *Bifidobacterium* spp.	
4	Cyclophosphamide	*Enterococcus hirae*, *Lactobacillus johnsonii*, *L. murinus*, *Barnesiella intestinihominis*	Th1 and Th17 immune response stimulation	Decrease in Clostridium cluster XIVa, *Roseburia, Lachnospiraceae*, *Coprococcus*, *lactobacilli*, and enterococci	
5	Oxaliplatin	Butyrate-producing bacteria	CD8+ T cells activation via ID2-dependent IL-12 signaling	Increased *Escherichia coli*, *Pseudomonas*, *Enterobacteriaceae*, and enterococci increased *Firmicutes/Bacteroidetes* ratio	
		Bacteroides fragilis and Erysipelotrichaceae	Increase in the anticancer effector/memory CD8+ T cells		

		Gram-negative bacteria with lipopolysaccharide component	Microbial lipopolysaccharide (LPS) interacted with TLR4 on macrophages results in hyperalgesia		
6	Cisplatin	Unspecified	MYD88-dependent signaling pathways alterations		
7	Floxuridine	*E. coli* and Comamonas	Efficacy is increased by *E. coli* whereas decreased by comamonas	Not specified	
8	Carmustine, etoposide, aracytine, and melphalan	Comamonas	Increased the efficacy of CPT	Increased *Proteobacteria*, decreased *Firmicutes and Actinobacteria*	
9	Gemcitabine	*Mycoplasma hyorhinis* and *Gammaproteobacteria*	Bacterial long isoform cytidine deaminase metabolized gemcitabine into its inactive form	*Proteobacteri*, *Verrucomicrobia*, *Akkermansia muciniphila*, *Escherichia coli*, *and Peptoclostridium difficile* (increased) *Firmicutes*, *Bacteroidetes*, *Bacteroidales*, *Lachnospiraceae*, *Ruminococcaceae (decreased)*	

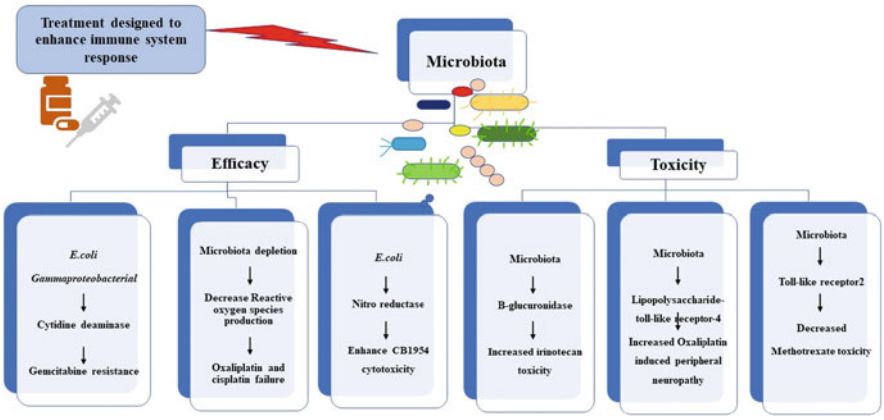

Fig. 2.2 Role of microbiota in efficacy and safety of chemotherapy

Fong et al. 2020). The gut microbiota plays an important role in functioning of host immune system and emerging evidence have shown effects of gut microbiota in various types of immunotherapies used in treatment of cancer. The use of therapies that target the gut microbiota is proving to be a viable way to improve cancer immunotherapy (Pouncey et al. 2018; Lee et al. 2021; Bhatt et al. 2017). Tumor cells evolve techniques to evade immunosurveillance, which would ordinarily recognize and remove them, according to one theory in cancer immunology. The role of microbiota in the efficacy and safety of immunotherapy is compiled in Table 2.5.

The microbiota has a significant role in immunity as well as in inflammation; therefore, its makeup can directly influence the responses of immunotherapy. The role of microbiota in the efficacy and safety of immunotherapy is presented in Fig. 2.3. CpG oligodeoxynucleotides are synthetic compounds that include unmethylated CG dinucleotides and mimic bacterial DNA and have been found to exhibit substantial immunostimulatory and anticancer action in several cancers (Narikiyo et al. 2004). The combined treatment (CpG oligodeoxynucleotides in combination with an antibody) have slow down the growth of tumor and increased survival of mice with EL4 lymphoma, MC38 colon carcinoma, and B16 melanoma (Iida et al. 2013). The well-known method is blocking of checkpoint inhibitors such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed death 1 (PD-1) (Seidel et al. 2018; Buchbinder and Desai 2016). Given the complex relationship between the gut microbiota and host immunity, accumulating data suggests that microbiota modification could help improve immunotherapy responses (Fig. 2.3). Rather than pharmacokinetics, commensal bacteria interact with ICIs via changing immunomodulation.

The importance of gut microbiota in immunotherapy efficacy has been demonstrated in preclinical trials (Table 2.6).

Table 2.5 Influence of immunotherapy on intestinal microbiota profiles (efficacy and toxicity) (Ting et al. 2022)

S No	Immunotherapy treatment	Microbes	Outcome
1	Anti-programmed cell death protein-1/ programmed cell death protein-ligand-1 mAbs	*Bifidobacterium longum*, *Collinsella aerofaciens*, *Enterococcus faecium*	FMT from R to germ-free mice enhances anti-2PD-L1 mAbs response with T cell enrichment
2	Monotherapy or combined immunotherapy	*Faecalibacterium prausnitzii*, *Coprococcus eutactus*, *Prevotella stercorea*, *Streptococcus sanguinis*, *Streptococcus anginosus*, *Lachnospiraceae bacterium*	L-rhamnose degradation, guanosine nucleotide biosynthesis
3	Anti-cytotoxic T lymphocyte-associated antigen-4 mAbs ± anti-PD-1	*Faecalibacterium prausnitzii*, *Bacteroides thetaiotaomicron*, *Holdemania filiformis*	R-enriched (combined anti-CTLA-4/anti-PD-1): R-enriched (anti-PD-1): Dorea formicigenerans
4	Anti-cytotoxic T lymphocyte-associated antigen-4	*Faecalibacterium*, *Gemmiger Clostridium*	R-enriched: XIVa. R-depleted: Bacteroides

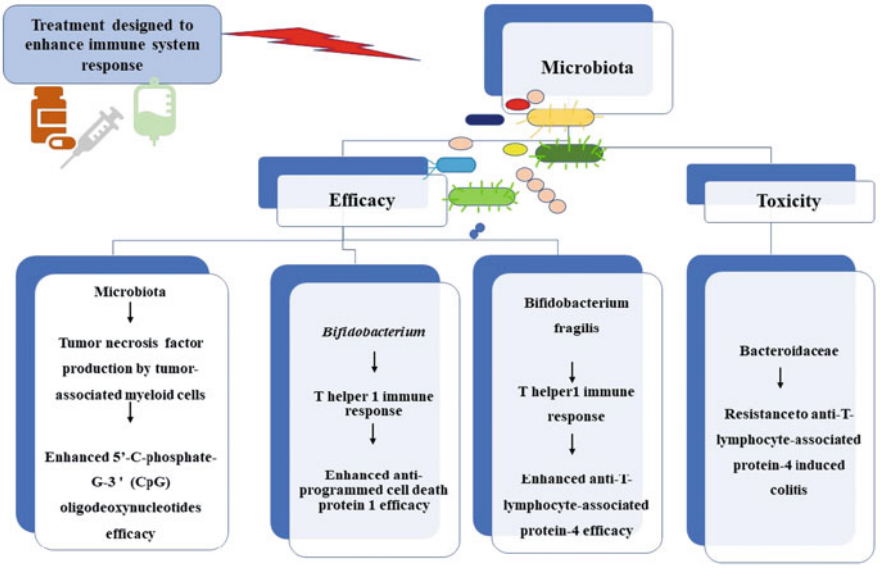

Fig. 2.3 Microbiota and immunotherapies (efficacy and toxicity)

Table 2.6 Available clinical trials related to cancer therapy and gut microbiome (Ting et al. 2022)

S No	Cancer type	Place	Global NCT number	Involvement	Outcome
1	Leukemia	France	02928523	Auto-FMT	Dysbiosis correction, eradication of multidrug-resistant bacteria, definition of dysbiosis, biosignature
2	Colorectal	Brazil	01609660	*Saccharomyces boulardii*	Mucosal cytokines, SCFA postoperative complications
3	Breast	Canada	03290651	Probiotics Natural Health Product-RepHresh Pro-B	Change in breast microbiota, inflammatory markers
4	Colorectal	Italy	00936572	*Probiotics*	Morphological and microbiological evaluation of the colonic microflora
5	Head-and-neck	Singapore	03552458	*Lactobacillus reuteri*	Oral mucositis severity, oral bacterial genetics, and transcriptional analysis
6	Colorectal	Finland	00197873	*L. rhamnosus* supplementation	Effect on treatment-related toxicity other than diarrhea
7	Colorectal	Sweden	03072641	(*Bifidobacterium lactis*, *L. acidophilus*)	Changes in microbiota composition and DNA methylation

2.5 Future Prospective

In cancer treatment, therapeutic resistance and toxicity are key roadblocks (Housman et al. 2014). There has been a lot of effort done into predicting therapy outcomes and optimizing treatment response. Gut microorganisms are obviously promising options for biomarkers and therapy targets. Despite the positive achievements so far, the future is not without its difficulties. A lack of mechanistic knowledge of the impact of microbiota changes on therapeutic response, undiscovered microbial profiles as biomarkers, and a lack of agreement on the best microbiota modulation technique are among these challenges (Veziant et al. 2021; Kashyap et al. 2017). Most of the research in cancers are focused on the bacteria; however, there is a role of other microorganisms also. Therefore, research is also needed to be conducted on commensal viruses, fungi, and archaea. To tackle the problems that lie ahead, concerted efforts are required. To deconstruct the biochemical intricacy of host–microbe–drug interactions, further functional investigations and prospective longitudinal human research are required (Miyoshi et al. 2020). Clinical translation requires a thorough understanding of the main microorganisms that influence therapy outcomes. As a result, interkingdom interactions of microbiota in cancer therapy is a promising future avenue (Merali et al. 2022; Vargason and Anselmo 2018).

Future clinical trials should evaluate the efficacy, long-term efficacy, and safety of various techniques, such as synbiotics, probiotics, prebiotics, and antibiotics. Many more secrets of the human microbiota are expected to be solved in future and our sincere thanks to all the researchers, clinicians, regulators, and patients for their time and efforts.

2.6 Conclusion

The microbiome holds enormous promise in cancer research. A rising amount of research links microbiome to anticancer therapy success in a bidirectional way, implying that these two elements can profoundly influence and control one another. As a result, the term "pharmacomicrobiomics" was coined to describe a new field that studies the interactions between medications and bacteria. The use of probiotics and antibiotics along with standard treatment could improve the efficacy and safety of anticancer drugs. Furthermore, certain concerns have been expressed about the safety of utilizing living microorganisms to manipulate the microbiota by probiotic supplementation. Therefore, further studies are urgently required to explore the individual profile of microbes which will help in the development of individualized microbiota manipulation tactics.

Despite these factors to consider, the data collected thus far on the enhancement of cancer therapy by microbiome alteration is both fascinating and encouraging. More research into the intricate network of interactions between medications, microbes, and the host could build an innovative path in the field of cancer.

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Synbiotics: Promising Approach for the Therapeutic Management of Cancer

3

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3.1 Introduction

Cancer is a disease represented by an uncontrolled growth and proliferation of cells due to evasion of central endogenous control mechanisms as well as the acquisition of metastatic properties. Typically, the upregulation of oncogenes and the downregulation of tumour suppressor genes can lead to dysregulated cell cycle progression and inhibited cell apoptotic mechanisms. In contrast to benign tumours, malignant tumours acquire metastasis, which involves the deactivation of cell adhesion receptors that are necessary for tissue-specific and cell to cell attachment, as well as the activation of receptors that induce cell motility. Activation of membrane metalloproteases also facilitates the spreading of metastatic tumour cells (Sarkar et al. 2013; Adjiri 2016). As such, managing cancer is highly challenging

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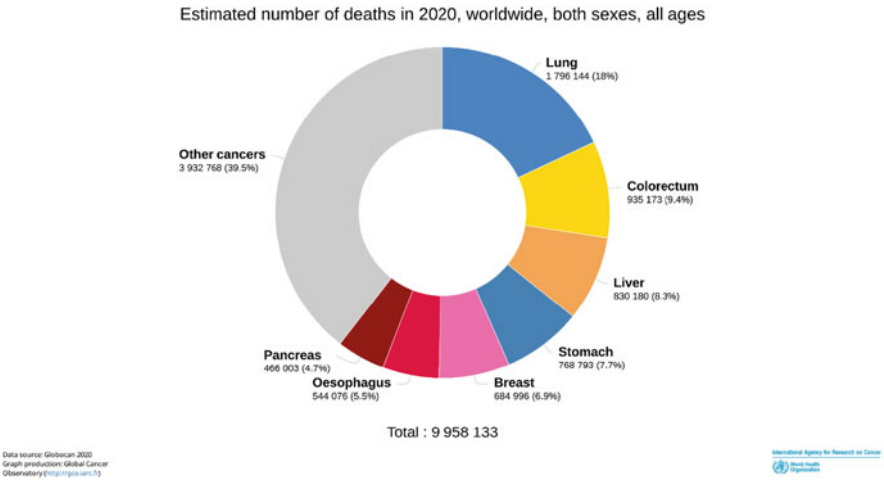

Fig. 3.1 Estimated number of cancer-related deaths worldwide in the year of 2020 for both sexes and across all ages. (Reproduced from Cancer Today, 2020 (https://gco.iarc.fr/today/online-analysis-pie?v=2020&mode=cancer&mode_population=continents&population=900&populations=900&key=total&sex=0&cancer=39&type=1&statistic=5&prevalence=0&population_group=0&ages_group%5B%5D=0&ages_group%5B%5D=17&nb_items=7&group_cancer=1&include_nmsc=1&include_nmsc_other=1&half_pie=0&donut=1), with permission from the International Agency for Research on Cancer, World Health Organization)

and complex, in which chemotherapy, radiotherapy, and surgery are the most common types of cancer treatment strategies available nowadays. These conventional cancer therapies have been documented throughout history by ups and downs not only due to their poor efficacy, therapeutic resistance, and associated adverse effects, but also in many cases, by hope and the reality of complete cancer remission and cure (Arruebo et al. 2011; Debela et al. 2021). Over the recent years, advancement in oncology research has also led to the discovery and development of novel therapeutic strategies such as immunotherapy, stem cell therapy, targeted therapy, chemodynamic therapy, sonodynamic therapy, and nanomedicine (Debela et al. 2021; Falzone et al. 2018; Chu et al. 2020). Despite that, cancer remains as one of the leading factors of death, and it is a significant barrier to an increased life expectancy across the world. According to the International Agency for Research on Cancer, World Health Organization (WHO), there was an estimated total of 9.9 million deaths globally in the year of 2020 with lung cancer being the leading factor of cancer-related deaths, responsible for nearly 1.8 million mortalities across both sexes (Fig. 3.1). The burden brought upon by cancer is expected to grow in the coming years, exerting immense emotional, physical, and financial strain on suffering individuals, their families, communities, as well as global health systems (WHO n.d., 2020; Sung et al. 2021). In addition, during the progression of cancer, tumours often become highly heterogeneous, thereby producing a mixed population of tumour cells that are characterized by varying molecular features and therefore, diverse responsivity to therapies and it is the primary factor responsible for the development of multiple resistant cancer phenotypes (Pucci et al. 2019). Although

huge resources have been devoted into oncology research, healthcare professionals and medical researchers are still struggling as most of the existing therapies are not entirely effective, and there is still a long journey ahead to a complete eradication of this disease that continues to kill numerous individuals each year (Adjiri 2016). Hence, a new revolution in the field of medical oncology is necessary not only to evade the development and/or progression of cancer, but also to optimize the efficacy and/or minimize adverse effects associated with existing anticancer therapeutics.

Every exposed human body surface such as the skin, as well as the respiratory, genitourinary, and gastrointestinal tracts are known to be heavily colonized by more than trillion of microorganisms that include fungi, archaea, bacteria, and viruses. Over the recent years, studies have identified commensal microorganisms as the key determinants of a host's homeostasis and health. For this instance, the gut microbiota is the most extensively populated, in which the human gastrointestinal tract is populated by a complex ecosystem of microorganisms that are commensal and undergo symbiotic co-evolution throughout the course of life of the host (Vivarelli et al. 2019; Markowiak and Ślizewska 2017). Generally, the gut microbiota represents a highly dynamic system whereby its density and composition can be influenced by exogenous factors such as environmental factors, drug use, diet, and infections, as well as endogenous factors such as genetic features of the host, age, and gender. Among these, diet has been evidenced to be key factor in dictating the composition and evolution of the gut microbiota over time (Ferraris et al. 2020; Hills et al. 2019). The production of advantageous micronutrients, strengthening gut integrity, harvesting energy, and modulation of a normal immunological response towards invading pathogens are among the essential functions exerted by beneficial intestinal microorganisms. However, there is potential for these functions to be disrupted due to an altered gut microbial composition, leading to the expansion of several microbiota subpopulations that produce high level of toxins (Hills et al. 2019; Thursby and Juge 2017; Vivarelli et al. 2019). As such, it has been widely proven that disruption in normal gut microbiota, known as microbiota dysbiosis, is implicated in the development of multiple chronic diseases ranging from inflammatory bowel disease, cardiovascular diseases, type 2 diabetes to various cancers (Hills et al. 2019; Olas 2020; Scott et al. 2018). The degree of gut microbiota dysbiosis is also direct causative factor in disease progression and prognosis (Shimizu et al. 2013). In terms of cancer, it is becoming increasingly well established that gut microbiota dysbiosis can contribute to almost all aspects of tumorigenesis ranging from susceptibility to cancer, cancer progression, development of co-infections, as well as the host's response to cancer therapeutics. Several microorganisms are also found to be specifically associated with cancer development and progression. For instance, *Helicobacter pylori* has been classified as a class I carcinogen by the WHO and plays a role in gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma. On the other hand, *Escherichia coli*, *Chlamydia trachomatis*, and *Salmonella enterica* are also linked to colorectal, cervical, and gallbladder cancers, respectively (Vivarelli et al. 2019; Khan et al. 2022). Therefore, achieving, restoring, and sustaining a favourable equilibrium in the gut environment, as well as the

activity of intestinal microorganisms is necessary to enhance the health condition of the host.

The old age quote by the father of modern medicine, Hippocrates, "Let food be thy medicine and medicine be thy food" represents the ideology of today's health-conscious population. There is a plethora of foods, plants, and herbs that has served as the backbone of traditional healing systems and has been integrated as part of global culture and history since the ancient times. Nutraceuticals, derived from "nutrition" and "pharmaceutical", are products with medicinal properties for disease prevention and treatment, as well as nutritional properties that aid in promoting physical health, maintaining proper body function, and improving longevity (Chan et al. 2021; Pandey and Naik 2015). Throughout these years, the roles of functional foods in maintaining good body health and the advantages of nutritional supplements in preventive healthcare have received increasing recognition and are being promoted around the world as a promising strategy to protect the body against various chronic diseases, in which extensive research have been focused on identifying and ascertaining health benefits of these products. Namely, probiotics and prebiotics are examples of nutraceuticals and functional foods that can positively alter, modify, and restore a normal gut microbiota (Pandey and Naik 2015; Alamgeer et al. 2018; Santana et al. 2016; Chanda et al. 2019). According to the Food and Agriculture Organization of the United Nations (FAO) and WHO, probiotics are defined as "live strains of strictly selected microorganisms which, when administered in adequate amounts, confer a health benefit on the host", whereas prebiotics are metabolic substrates defined as "non-digestible food supplements which exert advantageous health effects on the host through the induction of growth and/or activity of one or more gastrointestinal microorganisms" (Aponte et al. 2020). They are often utilized as substrates for probiotics, whereby galacto-oligosaccharides, fructo-oligosaccharides, and trans-galacto-oligosaccharides are the most utilized prebiotics currently. As such, synbiotics are essentially probiotics and prebiotics mixtures that are employed to synergistically influence the gut microbiota in which the prebiotic compound(s) can selectively favour the probiotic microorganism(s) (Davani-Davari et al. 2019; Davani-Davari et al. 2018). As a therapeutic strategy in cancer, synbiotics have the potential to exert onco-suppressive effects by repairing the gut microbiota and maintaining the native gut environment and intestinal barrier function, thereby improving health and overall human well-being. For instance, synbiotics can facilitate the detection and degradation of potential carcinogens and production of signalling molecules that influence cell death and proliferation. At the same time, synbiotics can prevent the conversion of non-toxic pro-carcinogen into toxic, harmful, and highly reactive carcinogens. Besides, synbiotics also influence the production of anti-inflammatory cytokines that can affect the process of tumorigenesis while activating phagocytes for the elimination of early-stage tumour cells (Scott et al. 2018; Górska et al. 2019; Gurry 2017; Raman et al. 2013). Moreover, when used as adjuncts in the treatment of cancer, synbiotics can also enhance the efficacy as well as minimize any adverse effects and complications brought upon by radiotherapy, chemotherapy, surgery, and other anticancer therapeutics (Scott et al. 2018; Shimizu et al. 2013). In this chapter, we

offer an overview into the potential anticancer properties of synbiotics. We also compiled some of the recent studies performed in this research area, which could serve as the basis for further research into the application of synbiotics as a novel therapeutic strategy and/or adjunctive therapy to existing therapeutics in the management of various cancers.

3.2 Potential Anticancer Properties of Synbiotics

Highlights

- Gut microflora dysbiosis predisposes an individual to cancer as it promotes tumour cells development, progression, and metastasis.
- An altered gut microbial composition induces genomic instability and mutations by genotoxins, promotes angiogenesis, sustains oncogenic pathways, as well as stimulates tumour-promoting inflammatory state by excessive production of inflammatory factors.
- Synbiotics modify the gut microbial ecosystem by increasing the population of beneficial microorganisms and reducing the population of harmful microorganisms.
- A healthy gut microflora also ensures mucosal integrity and normal cell processes, generating a protective microenvironment.
- A healthy gut microbiome primes the immune response, thereby promoting an anti-inflammatory and anti-oxidative state by regulating hormone levels and inducing immune infiltration into tumour cells.
- Synbiotics, when compared with prebiotics or probiotics alone, are more effective due to synergistic action in which prebiotic enhances colonization, growth, survival, and activity of probiotic strains.

3.2.1 Anti-inflammatory

Inflammation represents a natural reaction of the human immune system that can be stimulated by pathogens as well as infected cells and tissues. Therefore, inflammatory processes are part of the body defence mechanism that is essential in maintaining good health through the removal of injurious stimuli and facilitation of healing processes of damaged tissues (Greten and Grivennikov 2019; Chen et al. 2017). Typically, an inflammatory response is self-limiting, with its duration influenced by multiple molecules with dual anti-inflammatory and pro-inflammatory activities. As such, the resolution of an inflammatory response is a highly regulated process that involves spatially and temporally regulated secretion of mediators where chemokine gradients are diluted over time (Chen et al. 2017). Generally, the restoration of homeostasis involves the termination of tissue neutrophils infiltration, spent neutrophils apoptosis, counter-regulation of cytokines and chemokines, transformation of macrophages from classically to alternatively

activated cells, and lastly healing initiation. Effective resolution of inflammatory processes can prevent their progression into chronic inflammation, which ultimately prevents augmented damage of tissues and the progression of chronic inflammatory condition into chronic diseases (Chen et al. 2017; Neurath 2019). In the contrary, if an acute inflammatory response fails to restore tissue homeostasis, or if one or more steps of the inflammatory resolution process is/are dysregulated, it can lead to non-resolving and persistent chronic inflammatory process that is characterized by the presence of abnormal lymphocytes and macrophages which constantly secrete growth factors and cytokines (Chen et al. 2017; Zappavigna et al. 2020; Sugimoto et al. 2016).

In terms of cancer, it has been established that chronic inflammation can lead to cancer development and predisposes an individual to all stages of tumorigenesis (Greten and Grivennikov 2019). The relation between inflammation and tumorigenesis is generally driven through both intrinsic and extrinsic pathways. Intrinsic pathway is generally stimulated via biological alterations, which then lead to inflammation and invasive carcinoma. These transformations can include activation of proto-oncogenes, amplification, and rearrangement of chromosomes, as well as inactivation of tumour suppressor genes. An inflammatory microenvironment generated by inflammatory mediators from transformed cells has been reported to further promote the activation of oncogenes, damage of DNA and proteins, and the release of reactive oxygen species (ROS). In the contrary, extrinsic pathway is mediated via infections or inflammation which increase the chances for developing cancer in organs, such as the skin, pancreas, prostate, lung, and colon. Both intrinsic and extrinsic pathways interrupt tumour cells and modulate various signalling pathways. These include p53, mitogen-activated protein kinase (MAPK), and nuclear factor-kappa B (NF-κB), leading to an increased production of pro-inflammatory factors. These pro-inflammatory molecules further recruit and activate various leucocyte populations into the tumour microenvironment, whereby such a concerted action will lead to a more remarkable generation of inflammatory mediators and triggers a positive amplification loop that stimulates tumour growth and invasiveness (Greten and Grivennikov 2019; Multhoff et al. 2012; Zhao et al. 2021).

As mentioned earlier, the progression and development of cancer is typically a result of chronic inflammation aggravated by a lack of resolution to the underlying inflammatory processes (Hart et al. 2020). The body's homeostasis and tissue integrity are affected when tumours grow and metastasize, thereby signalling the body to initiate an acute phase inflammatory response. Several markers can be employed to detect the presence of inflammation and serve as prognosis indicator for chronic diseases including cancer. For instance, C-reactive protein (CRP) is the primary protein of the acute phase response reflecting the presence of tissue injury (Shrotriya et al. 2015). In general, the blood level of CRP reflects an ongoing inflammatory response and is frequently used as a minimally invasive indicator for several diseases including cancer. As such, the accumulation of CRP in blood often suggests the presence of an unresolved and advancing disease, such as cancer. For instance, high blood CRP level is directly correlated with the mortality of patients

with colorectal cancer, as well as hepatocellular carcinoma aggressiveness in patients with liver cancer. Besides, elevated levels of CRP in non-small cell lung cancer corresponds to tumour size and staging. In breast cancer, increased CRP levels are also associated with a higher mortality and declination of survival rate. For cancers involving the gastrointestinal tract, disease progression and advanced stage metastatic cancer with low survival were observed in patients with high blood CRP values (Hart et al. 2020; Shrotriya et al. 2015; Allin and Nordestgaard 2011; Fang et al. 2017; Xiao et al. 2019). Interleukin (IL)-6 is another example of major pro-inflammatory cytokines present in the tumour microenvironment. Dysregulation of IL-6 is often shown in tumours and overproduction of IL-6 has been discovered in almost every type of tumours, in which IL-6 overexpression is often observed in the tumour microenvironment suggesting a high correlation between carcinogenesis and inflammation. Namely, IL-6 enhances the process of tumorigenesis via modulation of specificities of tumours and various signalling pathways, which include survival, proliferation, apoptosis, angiogenesis, metastasis, invasiveness, as well as the metabolism of tumour cells. Furthermore, IL-6 facilitates the induction and restoration of countersignalling pathways thus shielding the cancer cells from therapeutic agents that induce apoptosis, oxidative stress, and DNA damage. As such, therapeutic agents that can potentially inhibit IL-6 and/or its associated signalling pathways presents great potential in cancer therapy (Kumari et al. 2016; Chonov et al. 2019).

Due to the strong correlation between inflammation and cancer, agents that can effectively suppress inflammatory responses may also be effective for the management of cancer. For instance, there have been several research that demonstrated the potential of synbiotics in suppressing inflammatory responses.

- A double-blind, randomized controlled trial evaluated the impact of administering preoperative synbiotic in colorectal cancer patients subjected to colorectal resection. Patients were randomized to either the synbiotics group where Simbioflora comprising the probiotics *Lactobacillus rhamnosus, Lactobacillus acidophilus*, *Bifidobacterium lactis*, and *Lactobacillus casei*, as well as 6 g of fructo-oligosaccharide were given to patients preoperatively or placebo group where patients received maltodextrin. The study was carried out for a duration of 7 days and all patients received nutritional assessments and evaluation of CRP and IL-6 levels. Results showed that IL-6 (163.2 ± 19.5 versus 138.8 ± 12.5) and CRP levels (10 ± 5.2 versus 7.17 ± 3.2) were decreased significantly in the group taking synbiotic after 7 days. There were no substantial changes seen in the placebo group for IL-6 levels (154.2 ± 18.3 versus 160.9 ± 18.6) and CRP (10.6 ± 6.18 versus 10.4 ± 6.1). This study concluded that oral synbiotics supplementation given to patients preoperatively managed to attenuate the body's inflammatory state (Polakowski et al. 2019).
- One study assessed the impact of perioperative oral supplementation of synbiotics upon systemic inflammatory responses in patients undergoing high-risk hepatobiliary resection. One hundred and one biliary cancer patients with planned combined liver and extrahepatic bile duct resection with hepaticojejunostomy were randomly assigned to group A, where patients received enteral feeding with

synbiotics after the operation, or group B, where they received synbiotic treatment before and after the operation. The synbiotic supplementation consisted of one 100-mL bottle of Bifiel containing *Bifidobacterium breve* strain Yakult, one 80 mL bottle of Yakult 400 containing *Lactobacillus casei* strain Shirota; and 15 g/day of galacto-oligosaccharides. Group B patients received synbiotics administration for continuously 2 weeks before hepatectomy, whereby patients in Group A did not. It was found that preoperative IL-6 levels of patients in group B decreased significantly while levels of IL-6 in group A patients remained unchanged (5.1 vs 11.0 pg/dL). White blood cell counts, CRP levels and postoperative serum IL-6 levels of patients in group B showed a significant reduction when compared to those of group A patients. Thus, this study concluded that synbiotics can alter systemic inflammatory responses (Sugawara et al. 2006).

- A double-blind, randomized controlled trial was performed to evaluate the effect of synbiotics on inflammatory markers in type 2 diabetes mellitus patients. 44 subjects were randomly assigned to take either one synbiotic tablet daily or one placebo tablet daily for 8 weeks. It was found that there was a substantial reduction in the serum CRP levels (4.15 ± 1.96 vs 4.94 ± 2.36 mg/L), IL-6 (8.12 ± 5.02 vs 9.19 ± 5.97 ng/L), and tumour necrosis factor (TNF)-α (7.36 ± 2.61 vs 8.03 ± 2.73 ng/L) in patients receiving synbiotic at the end of 8 weeks when compared with baseline. Such a reduction was highly significant in contrast to the placebo group, in which no remarkable changes were reported in the placebo group. These findings indicate that synbiotic supplementation can reduce serum markers of inflammation (Akram Kooshki et al. 2015).
- A double-blind, randomized controlled trial assessed the potential of synbiotic in improving inflammatory markers and gastrointestinal wellness in middle-aged individuals. The researchers demonstrated that synbiotic containing *Bifidobacterium animalis* subsp. *lactis* and fructo-oligosaccharides decreased inflammatory status in these subjects. In the study, individuals were randomly assigned to take either placebo or synbiotic for 30 days and the levels of plasma pro-inflammatory cytokines were evaluated. It was showed that the levels of interferon (IFN)-γ, IL-8, IL-6, and IL-17a were remarkably lowered after synbiotic supplementation for 30 days which effects were not observed in the placebo group and were independent of gut barrier function. Notably, IL-6 was particularly suppressed among other pro-inflammatory markers (Neyrinck et al. 2021).
- An in vivo study examined the anti-inflammatory potential of preventive administration of synbiotics containing *Lactobacillus plantarum* and inulin in chronic inflammatory rats upon *N*,*N*-dimethylhydrazine administration. It was showed that administration of *N*,*N*-dimethylhydrazine stimulated the production of various pro-inflammatory mediators, including cyclooxygenase (COX)-2, inducible nitric oxide synthase (iNOS), and NF-κB, and the pro-inflammatory cytokines IL-2, IL-17, IL-6, and TNF-α. Notably, these inflammatory processes in the jejunal mucosa were attenuated upon 28 weeks supplementation with synbiotic and the synthesis of the anti-inflammatory cytokine IL-10 was stimulated. These findings indicate that synbiotic can suppress chronic inflammation by

downregulating various inflammatory factors and their underlying signalling pathways (Štofilová et al. 2015).

In short, the above studies clearly demonstrated the positive impact of synbiotics in attenuating the body's inflammatory states. Given the close relationship between inflammation and cancer, synbiotics have great potential for application in cancer prevention and treatment.

3.2.2 Anti-oxidative

Oxidative stress arises when the production of reactive metabolites and free radicals such as hydroxyl radicals, hydrogen peroxide, superoxide radicals, and singlet oxygen collectively known as ROS, exceeds the detoxification ability of the body on these reactive products (Pizzino et al. 2017). ROS are redox messengers that are generated as metabolic by-products by the biological system when there is a partial reduction of molecular oxygen. Several key biological processes including the activation of transcriptional factors, protein phosphorylation, cell differentiation and apoptosis, as well as immunity rely on well-regulated production of ROS in which the presence of ROS should be kept at a low but optimal level within cells. When ROS is present at low levels, their negative effects can be safely neutralized by the body's natural antioxidant defences. Among which, catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) are examples of enzymes responsible for protecting cells from the deleterious effects of ROS (Pizzino et al. 2017; Bhattacharyya et al. 2014). Conversely, when normal cellular homeostasis could not cope due to an excessive accumulation of ROS, it can result in oxidative stress and cellular dysfunction. Namely, a prolonged and long-term exposure to pro-oxidant factors can induce structural defects at the level of mitochondrial DNA while altering the functions of enzymes and cellular structures, resulting in aberrant gene expression. Thus, oxidative stress is often responsible for the initiation and progression of various chronic diseases such as respiratory diseases, cardiovascular diseases, neurodegenerative diseases, diabetes, and cancer (Sharifi-Rad et al. 2020).

In terms of cancer, oxidative stress influences with all three major stages of the disease, namely, initiation, promotion, and progression. During the initiation stage, excessive ROS may induce DNA damage through the introduction of structural alterations and gene mutations. Namely, ROS can result in an abnormal gene expression to modulate second messenger systems and cell-to-cell communications during the promotion stage (Reuter et al. 2010). For instance, the oxidation of negative feedback loop controllers can be attributed to an increased ROS level, thereby dictating the behaviour of multiple signalling pathways underlying the growth of tumours and programmed cell death via the MAPK and phosphoinositide 3-kinase (PI3K)/protein kinase B (PKB) pathways, which leads to the induction of cell proliferation and reduced apoptosis of the initiated cell population. Besides, the accumulation of ROS in tumour cells also leads to the downregulation of phosphatase and tensin homolog (PTEN), which in turn upregulates PI3K/PKB signalling

that further induces tumour cells proliferation (Arfin et al. 2021; Saha et al. 2017). Finally, the rise in ROS levels may exploit the underlying mutagenesis and genomic variability in tumour cells to stimulate the progression of cancer. Further DNA alterations may also be added to the initiated cell population, thereby accelerating the progression of the disease (Reuter et al. 2010; Arfin et al. 2021; Saha et al. 2017). Apart from that, ROS is also involved in the crosstalk between chronic inflammation and cancer. One important feature of tumour promoters is their capability to recruit and activate inflammatory cells while inducing them to generate ROS. Therefore, such an accumulation of inflammatory cells within the tumour microenvironment can result in an overproduction of ROS through the activation of oxidant-generating enzymes, such as iNOS, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, and myeloperoxidase, as well as the stimulation of lipoxygenase (LOX) and COX-2 (Reuter et al. 2010; Aggarwal et al. 2019). At the same time, ROS can influence the type, presence, and the levels of inflammatory factors such as peroxisome proliferator-activated receptor (PPAR)-γ, NF-κB, activator-protein (AP)-1, p53, inflammatory chemokines and cytokines, hypoxia-inducible factor (HIF)-1α, as well as growth factors. Thus, the abundant accumulation of ROS along with inflammation-modulating factors causes an elevation in mutation load, signal transduction defects, and apoptosis deactivation (Aggarwal et al. 2019). In addition, increased ROS also facilitates tumour cell angiogenesis and metastasis. Studies have shown that ROS can promote the generation of new blood vessels to improve oxygen and nutrient supplies for meeting increased metabolic needs of proliferating tumour cells. As for tumour cells metastasis, it has been documented that ROS promote epithelial mesenchymal transition by stimulating the activity of matrix metalloproteinases that mediate proteolytic degradation of components of the extracellular matrix (Aggarwal et al. 2019; Aboelella et al. 2021). In a nutshell, oxidative stress is undoubtedly a major influence of the hallmarks for cancer development. Hence, reduction and/or attenuation of oxidative stress represents one of the vital strategies in the prevention and/or treatment of cancer.

Evidences have affirmed the anti-oxidative properties of synbiotics, in which several studies have documented a decrease in ROS and/or other oxidative stress markers upon synbiotics supplementation.

- One study assessed the impact of synbiotic supplementation consisting of the probiotic bacteria *Lactobacillus casei* and inulin prebiotic on the antioxidant properties of human plasma. Thirty-two healthy volunteers were randomized to either control or synbiotic groups, and their blood samples were collected prior to synbiotic supplementation and after 7 weeks at the end of the study. Antioxidant markers, namely, GPx activity, SOD, CAT, and the ferric reducing ability of plasma (FRAP) in human plasma were measured before and after synbiotics supplementation. Generally, SOD, CAT, and GPx are antioxidant enzymes that can neutralize oxidants in the intestinal tract whereas FRAP is a sensitive indicator of the biological fluid's total antioxidant status. SOD can be found in three forms, namely, manganese SOD, copper-zinc SOD, and extracellular SOD. It

catalyses the reaction of superoxide anion dismutation and such activity shields cells from oxidative stress. Another antioxidant enzyme that interacts closely with SOD is CAT. CAT decomposes hydrogen peroxide into oxygen and water. The reduction of CAT activity is often seen in oxidative stress-induced diseases including cancer. GPx acts by reducing hydrogen peroxide and organic peroxides in the presence of reduced glutathione (GSH). Results from the study showed that the FRAP values and CAT activity were increased significantly in the synbiotics group following synbiotics administration and the increase in SOD and GPx activity is insignificant compared to controls. The study concluded that synbiotics may have a positive impact on the activity of selected antioxidant enzymes and human plasma antioxidant capacity (Kleniewska et al. 2016).

- One study assessed the effect of synbiotic containing *L. casei* and inulin on the parameters of oxidative stress, such as hydrogen peroxide, concentrations of malondialdehyde (MDA), free sulfhydryl groups content, and glutathione. Thirty-two healthy volunteers were randomized to either control or synbiotic groups. One capsule of synbiotic was provided to the synbiotic group every day for 7 weeks. Blood sample of the subjects were collected prior to synbiotic supplementation and after 7 weeks at the end of the study (Kleniewska and Pawliczak 2017). MDA is an important marker of antioxidant status and oxidative stress in patients with cancer. It is one of the final products of polyunsaturated fatty acid peroxidation in cells and its overproduction is the result of increased free radicals (Ayala et al. 2014). Glutathione, on the other hand, is a major antioxidant in the body in which glutathione disulphide (GSSG) represents the oxidized form of glutathione. The levels of GSSG often increase when there is an increase in intracellular oxidative stress associated with disease states (Sadhu et al. 2016). Results showed that there is a significant decrease in MDA, hydrogen peroxide, and GSSG concentrations in the synbiotic group in contrast to control, as well as a remarkable increase in the concentrations of total glutathione, GSH, and free sulfhydryl (-SH) group content in the synbiotic group in contrast to control. Thus, this study concluded that synbiotics could positively impact selected markers of oxidative stress (Kleniewska and Pawliczak 2017).
- One double-blind, randomized controlled trial assessed the impact of synbiotics supplementation on oxidative markers in breast cancer survivors. Eighty-eight subjects were randomized to take either synbiotic supplement containing *L. rhamnosus*, *L. casei*, *L. bulgaricus*, *L. acidophilus*, *S. thermophilus*, *B. longum*, *B. breve*, and fructo-oligosaccharides, or a placebo for 10 weeks. GPx, MDA, SOD concentration, and serum total antioxidant capacity were evaluated at the end of the study. It was demonstrated that serum MDA levels were remarkably reduced whereas serum SOD concentration was significantly increased after 10 weeks of synbiotic supplementation in contrast to placebo (Navaei et al. 2020).
- One study has reported that synbiotics present greater antioxidant properties than prebiotics alone. The researchers prepared yogurt with either the probiotics *L. fermentum* and *L. plantarum* alone, or with the prebiotic fructo-oligosaccharides. It was demonstrated that the supplementation of fructo-

oligosaccharides promoted the growth of both probiotic strains. Besides, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was also remarkably greater in synbiotic yogurt with a highest total phenolics and ferric reducing power as compared with prebiotic and control yogurt. Such findings suggest that application of synbiotics as an antioxidant may offer added advantages due to the synergistic effect of both probiotics and prebiotics (Madhu et al. 2012).

To sum up, the above studies demonstrated that synbiotics can shield the body from oxidative stress. Since oxidative stress is closely related to cancer, synbiotics can be considered for application in cancer prevention and/or treatment.

3.2.3 Immunomodulatory

The human immune system and cancer are closely related, and its link has been widely acknowledged over the last decade. The basis for the connection between immunity and cancer involves three fundamental principles on defence mechanism of our immune system and how it can protect an individual (Pardoll 2015; Pandya et al. 2016). Namely, the immune system detects foreign antigens from malignant cells or pathogens, possesses effector functions that can specifically target and eliminate malignant cells or pathogens for host protection, and activates the adaptive immune response to develop immunological memory for subsequent defence mechanisms. Such a multifaceted mechanism provides effective immune surveillance that can contribute to elimination of any attack against the host or injury. For this instance, the ability of tumour cells to evade immune surveillance represents one of the most established cancer hallmarks, thereby giving rise to the paradigm of treatments within the context of immunomodulation (Pandya et al. 2016; Hoos and Britten 2012; Gonzalez et al. 2018).

As discussed earlier, it is known that the gut microbiota has a profound influence on developing a plethora of diseases which include cancer. The gut microbiota regulates human health and primes the body's immune system by regulating both systemic and local immune responses. Therefore, the manipulation of the gut microbiota is a great strategy in cancer prevention and/or treatment as they share a close relationship (Inamura 2021). Generally, local immune responses are triggered by microbes via immune cells interactions that express pattern recognition receptors (PRR) such as Toll-like receptors (TLR). Local dendritic cells (DC) are also activated by microbes via interactions with PRR. Naive T cells' differentiation into effector T cells is induced by the activated DCs, particularly, T helper cells and regulatory T cells when they travel from the gastrointestinal tract to the mesenteric lymph nodes where microbe-derived antigens were presented. Local immune response occurs when a subset of effector T cells travels back to the gastrointestinal tract whereas systemic immunity occurs when the remaining effector T cells enter the systemic circulation. Regulatory T cells release anti-inflammatory cytokines including IL-10, as well as tumour growth factor which will transform the

pro-inflammatory state of the immune system into an anti-inflammatory state. In the contrary, T helper cells transform the immune system from an anti-inflammatory state into a pro-inflammatory state through the secretion of immunostimulatory cytokines such as IL-17, or through the recruitment and activation of neutrophils (Inamura 2021; Ma et al. 2019; Pennock et al. 2013; Kumar et al. 2018; Mellman 2013). Natural killer cells are cytotoxic towards primary tumour cells and inhibits metastasis by preventing proliferation, migration, and colonization of tumour cells to distant tissues. Moreover, natural killer cells modulate adaptive immune responses in the body by producing a large number of cytokines, mainly IFN-γ. Natural killer cells are also specific in antitumour cytotoxicity as they could differentiate healthy cells from abnormal cells and thus reducing off-target complications (Wu et al. 2020). In short, DCs, natural killer cells and T cells are the key effectors for the timely detection and deletion of damaged and potentially carcinogenic cells. Hence, increasing beneficial bacteria in the gut via synbiotics supplementation is important for maintaining a normal host defence mechanism as intestinal microflora is vital in providing a natural defence against invading pathogens.

Ideally, probiotics act to increase the population of beneficial microorganisms whereas prebiotics act to boost the formation of resident beneficial intestinal bacteria. Prebiotics provide food for the swallowed probiotics, keeping the flora from being depleted. Dietary carbohydrates that escape digestion/absorption in the small bowel, as well as prebiotics, ferment in the colon, producing short-chain fatty acids that help *Lactobacilli* and *Bifidobacteria* thrive. There have been studies that documented the positive effects of synbiotics in modulating immune responses.

- A randomized study evaluated the impact of a synbiotic containing the probiotic *Lactobacillus casei* with the prebiotic dextran on humans and mice, respectively. For the study involving human subjects, eight healthy adult volunteers received lyophilized *L. casei* and dextran once per day for 7 days. Isolation of peripheral blood mononuclear cells (PBMC) from heparinized venous blood was performed on days 0, 8, and 11 and natural killer cells activities were measured. Results showed that the natural killer cells activity increased significantly from 28.7 ± 9.8% on day 0 to 43.9 ± 15.9% on day 8 (1 day post-supplementation) and 41.7 ± 13.2% on day 11 (4 days post-supplementation). Notably, it was reported that the percentage of natural killer cells in the peripheral blood lymphocyte fractions has the potential for continual increment post-supplementation. These results indicated that oral supplementation of synbiotics increases the ability and number of natural killer cells in humans. The study also investigated the anti-tumour activities of the synbiotic on murine models. BALB/c mice were assigned into four different groups, namely, the synbiotic group, probiotic group, prebiotic group, as well as control. Meth A cells were inoculated intraperitoneally into all mice and the survival rate was tracked for up to 80 days. It was found that the survival rate of the prebiotic and probiotic groups was 33.3%, whereas the control group showed survival rate of only 8.3%. Additionally, the synbiotic group demonstrated remarkable elevation in survival rate of 50% as compared to all the other groups. These results proved that synbiotic supplementation

remarkedly elevated anti-tumour activities. In short, the results of both human and animal experiments indicated that host immune functions were enhanced, and antitumour activities were boosted with synbiotics (Ogawa et al. 2006).

- One double-blind, randomized controlled trial has assessed the impact of synbiotic on faecal microbiota and immunity of healthy elderly individuals. Subjects were assigned to consume either synbiotic containing *L. rhamnosus* and soluble corn fibre, or placebo for 3 weeks. Faecal microbiota analyses showed that the synbiotic greatly increased *Parabacteroides* spp. while decreased *Oscillospira* spp. and *Desulfovibrio* spp. This indicated that synbiotic supplementation can facilitate reduction in the population of harmful microorganisms, as *Desulfovibrio* spp. represents a group of sulphate-reducing bacteria that has been implied as one of the key players in the pathogenesis of various gastrointestinal diseases, including colon cancer. Besides, it was also observed that synbiotics consumption enhanced natural killer cells' activity in contrast to control. A positive immunomodulatory effect was further evidenced by a remarkable decline in the level of pro-inflammatory cytokine IL-6, indicating that synbiotic supplementation can be an attractive option for positively enhancing the immune system against various diseases including cancer (Costabile et al. 2017).
- An in vivo study evaluated the potential of synbiotics in altering the colonic microbiome and modulating gastrointestinal immune and inflammatory responses on a spontaneous colitis mice model of inflammatory bowel disease. The combined and individual efficacies of the probiotic *Bacillus coagulans* and the prebiotic sugarcane fibre were evaluated in the study. Gut microbiota dysbiosis, specifically the depletion of both *Bacteroidetes* and *Firmicutes*, has been associated with the development of gastrointestinal diseases. As such, it was found that synbiotic supplementation increased both the levels of *Bacteroidetes* and *Firmicutes*, thereby reversing dysbiosis of the gut microbial community. Besides, the genus *Prevotella* belonging to the phylum *Bacteroidetes* was also enhanced. Such finding was consistent with established evidence that a high fibre diet increases prevalence of *Prevotella*, which is a dietary fibre fermenter for the production of short-chain fatty acids, indicating that the presence of prebiotic within the synbiotic formulation presented synergistic effect in addition to the benefits of probiotic. The results further documented that both probiotic and prebiotic exhibited immunomodulatory effects, however, synbiotic supplementation had the greatest effect in modulating the overall immune profile, namely, innate immune system activation (Shinde et al. 2020).
- One double-blind, randomized controlled trial evaluated the influence of synbiotics on intestinal microbiota and the negative impact of chemotherapy oesophageal cancer patients receiving neoadjuvant chemotherapy. The subjects were randomized to either the group taking synbiotic, where subjects were given 3 g/day of Yakult BL Seichoyaku containing *Bifidobacterium breve* strain Yakult and *Lactobacillus casei* strain Shirota, and 15 g/day of galacto-oligosaccharides, or the control group where they were only given 3 g/day of Biofermin containing *Streptococcus faecalis*. Results showed that on the tenth day of chemotherapy, the number of beneficial bacteria were significantly larger while harmful bacteria

were smaller. Concentrations of propionic acid and acetic acid were also remarkably greater in subjects receiving synbiotic. It was also observed that the occurrence of diarrhoea and severe lymphopenia were reduced significantly. It was concluded that intestinal microbiota could be manipulated by synbiotic supplementation, which is beneficial to cancer patients undergoing chemotherapy in terms of minimized adverse reactions (Motoori et al. 2017).

Given the prominent correlation between the gut microbiota and the human immune system and subsequent oncogenesis, a normal gut microbiota equilibrium is crucial for immune system maturation and to prime an anticancer response. As such, manipulation of the gut microbiota is an advantageous approach in preventing or treating cancer. The microbial ecosystem in the gut can be modified through synbiotic supplementation, in which the beneficial microbes in the gut and their survival can be improved, thereby providing health benefits to the host.

3.3 Application of Synbiotics in the Management of Cancer

As discussed above, synbiotics may present positive effects in the management of cancer due to their ability to produce anti-inflammatory, anti-oxidative and immunomodulatory effects. Apart from that, perioperative probiotics, prebiotics, and/or synbiotics supplementation have also been found to contribute to reduced complications, adverse effects, and overall quality of life in cancer patients undergoing treatment. In this section, we have selected some notable studies that demonstrated the potential of probiotics, prebiotics, and synbiotics in alleviating different cancers, their complications, and/or adverse reactions from the use of existing anticancer therapeutics. The key findings from these studies are summarized in Table 3.1.

3.4 Conclusion and Future Directions

The rising prevalence of cancer across the world has prompted the need for the search and development of effective therapies in eradicating the disease. As the causal relationship between cancer and gut microbiota dysbiosis has been observed in various studies, synbiotics have been proposed as the promising agent to restore gut homeostasis and generate a protective microenvironment against various cancers. Nevertheless, the exact differences of the gut microbiota between normal and tumour cells at each step of cancer development and progression is still not yet fully elucidated. Therefore, deciphering the key manifestations of dysbiosis may help researchers to better understand the discrepancies in cancer progression and its response to therapies in patients with similar clinical profiles. Although there have been multiple studies employing probiotics, prebiotics, and/or synbiotics for managing cancer over these years, they are mostly used as adjuvant in addition to existing anticancer strategies as an attempt to minimize adverse reactions and treatment

Table 3.1 Summary of primary findings from studies employing probiotics, prebiotics, and synbiotics for managing different types of cancers

Type of cancer	Type of study	Duration of supplementation	Content	Key findings	Reference
Biliary cancer	Randomized controlled clinical trial: Biliary cancer patients involving hepatic hilus undergoing hepatectomy	2 weeks preoperative	*L. casei*, *B. breve*, galacto-oligosaccharides	• Lymphocyte counts and natural killer cells activity were increased preoperatively while IL-6 was suppressed in patients given synbiotics. • Total organic acid concentrations and the numbers of cultured *Bifidobacterium* colonies and increased significantly in faeces of preoperative patients given synbiotics. • Concentration of acetic acids and total organic acids showed remarkably elevation in faeces of postoperative patients given synbiotics. • White blood cell counts, CRP, and serum IL-6 were remarkably lower postoperatively in patients given synbiotics. • Overall incidences of postoperative infectious complications were 12.1% in patients given synbiotics in contrast to 30.0% in patients of the control group.	Sugawara et al. (2006)

	Randomized controlled clinical trial: Biliary cancer patients undergoing hepatectomy	14 days postoperative	*B. breve*, *L. casei*, galacto-oligosaccharides	• Synbiotics modified faecal microflora of surgical patients. • Numbers of beneficial microorganisms such as *Lactobacilli* and *Bifidobacteria* increased while harmful microorganisms such as *Pseudomonas*, *Enterobacteriaceae* and *Candida* decreased postoperatively in the group taking synbiotic in contrast to control. • Total concentration of organic acids increased in the group taking synbiotic in contrast to control. • Overall infectious complications incidence was 19% in the synbiotic group in contrast to 52% in the control group.	Kanazawa et al. (2005)
Breast cancer	Double-blind, randomized controlled clinical trial: Overweight and obese breast cancer survivors	10 weeks	*L. acidophilus*, *L. casei*, *B. longum*, *L. rhamnosus*, *B. breve*, *L. bulgaricus*, *S. thermophilus*, fructo-oligosaccharides	• Synbiotic supplementation led to a remarkable decline in serum MDA levels as compared with placebo. • Remarkably increased the concentration of serum SOD in the group taking synbiotic in contrast to control.	Navaei et al. (2020)

(continued)

Table 3.1 (continued)

Type of cancer	Type of study	Duration of supplementation	Content	Key findings	Reference
	Double-blind randomized controlled clinical trial: Breast cancer survivors with lymphedema	10 weeks	*L. acidophilus*, *L. casei*, *B. longum*, *L. rhamnosus*, *B. breve*, *L. bulgaricus*, *S. thermophilus*, fructo-oligosaccharides	• Synbiotic supplementation resulted in a remarkable decline in leptin and TNF-α in contrast to the placebo group. • Significantly reduced oedema volume within the synbiotic group post-intervention.	Vafa et al. (2020)
	In vitro: 4T1 breast cancer cells; in vivo: Female BALB/c mice administered with 4T1 tumour cells	In vitro: 72 h; In vivo: 28 days	Kefir water: *L. acidophilus*, *L. casei*, *L. lactis* (No prebiotic)	• Kefir water was cytotoxic towards 4T1 tumour cells with an IC_{50} value of 12.5 and 8.33 mg/mL at 48 and 72 h, respectively. • Animals treated with Kefir water presented remarkable suppression in weight and size of tumour. • Remarkable fivefold increment in T helper cells and sevenfold increment in cytotoxic T cells were reported in the Kefir water group. • Significant reduction in pro-inflammatory and pro-angiogenic markers were reported in the Kefir water group.	Zamberi et al. (2016)

	In vivo: *N*-methyl-*N*-nitrosourea-induced mammary carcinogenesis in female Sprague-Dawley rats	16 weeks	*L. plantarum*, oligofructose-enriched inulin	• Remarkable reduction in high to low-grade carcinomas ratio and in the expression of Ki-67 were observed upon synbiotic treatment. • Synbiotic enhanced the infiltration of CD8+ and CD4+ T cells into the tumour. • Synbiotic upregulated CD25+FoxP3+ regulatory T cells in tumours. • Synbiotic administration presented anti-proliferative and immunomodulatory activities.	de Vries et al. (2006)
	Randomized controlled clinical trial: Overweight and obese women with breast cancer-related lymphedema	10 weeks	*L. acidophilus*, *L. casei*, *B. longum*, *L. rhamnosus*, *B. breve*, *L. bulgaricus*, *S. thermophilus*, fructo-oligosaccharides	• Significant improvement in quality of life by 39.53% in the group taking synbiotics in contrast to only 1.5% in control. • Remarkable reduction in body mass index by 3.01% in the group taking synbiotic in contrast to control. • Oedema volume reduced remarkably in the group taking synbiotics in contrast to control.	Vafa et al. (2020)
	Triple-blind, randomized controlled clinical trial: Overweight and obese breast cancer survivors	8 weeks	*L. acidophilus*, *L. casei*, *B. longum*, *L. rhamnosus*, *B. breve*, *L. bulgaricus*,	• Significant decline in serum insulin and insulin resistance were observed in the group taking synbiotics.	Raji Lahiji et al. (2021)

(continued)

Table 3.1 (continued)

Type of cancer	Type of study	Duration of supplementation	Content	Key findings	Reference
			S. thermophilus, fructo-oligosaccharides	• Reducing effects on glycaemic profile, IGF-1, and sex hormones was observed; however, these effects were insignificant.	
Colorectal cancer	Double-blind, randomized controlled clinical trial: Colorectal cancer patients subjected to colorectal resection	Preoperative: 7 days; Postoperative: 30 days	*B. lactis*, *L. rhamnosus*, *L. acidophilus*, *L. casei*, fructo-oligosaccharides	• Significant suppression in the levels of serum IL-6 and CRP were observed in the synbiotic group after 7 days in contrast to control. • Infectious complications occurred postoperatively in 2.8% of patients receiving synbiotics in contrast to 18.9% in control. • Mean antibiotic use time in the synbiotic group was 1.42 ± 0.5 days compared to 3.74 ± 4.3 days in control. • Mean hospital stay length in the synbiotic group was 3 ± 1 days compared to 4 ± 18 days in control. • No death was reported among patients receiving synbiotics; however, three deaths were reported in control.	Polakowski et al. (2019)

	Double-blind, randomized controlled clinical trial: Colon cancer patients and polypectomized patients	12 weeks	*B. lactis*, *L. delbrueckii*, oligofructose-enriched inulin	• Synbiotic supplementation significantly increased *Lactobacillus* and *Bifidobacterium*, whereas *Clostridium perfringens* reduced in faecal flora. • Synbiotic remarkably suppressed colorectal proliferation and the capacity of faecal water to stimulate necrosis in colonic cells. • Polypectomized patients receiving synbiotics had improved epithelial barrier function. • Genotoxicity assays of colonic biopsy samples of polypectomized patients revealed reduced exposure to genotoxins after 12 weeks. • Synbiotic suppressed the production of IL-2 by peripheral blood mononuclear cells. • Synbiotic induced IFN-γ production.	Rafter et al. (2007)
	In vivo: 1,2-dimethylhydrazine dihydrochloride-induced experimental colon cancer in male Wistar rats	7 weeks	*L. casei*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *Bifidobacterium* spp., *S. thermophilus*, *B. longum*, fructo-oligosaccharides	• Animals that received synbiotic had 100% survival as compared with only 70% survival in animals that received only carcinogen. • Animals that receive	Vagianos et al. (2018)

(continued)

Table 3.1 (continued)

Type of cancer	Type of study	Duration of supplementation	Content	Key findings	Reference
				synbiotics had remarkably lower percentage of colitis-like lesions, aberrant crypt foci, dysplasia, and inflammation. • Least pathological lesions in animals that received synbiotics from the beginning of the study (7 weeks) in contrast to the animals which only received synbiotics for 5 weeks.	
	In vivo: 1,2-dimethylhydrazine-induced colonic tumorigenesis model of male ICR mice	24 weeks	*B. longum*, *L. gasseri* (No prebiotic)	• Reduced colon tumour multiplicity and the size of the tumours. • Reduced colonic mucosa cellular proliferation rate and inhibited tumour induction by 1,2-dimethylhydrazine. • Significantly increased phagocytic activity of peritoneal macrophages. • Remarkable upregulation of RAW264.7 macrophages proliferation due to an increased S phase DNA synthesis. • Upregulated cyclin A and proliferating cell nuclear antigen.	Foo et al. (2011)

	In vivo: Azoxymethane-induced experimental colon cancer in male Fischer 344 rats	33 weeks	*L. rhamnosus*, *B. lactis*, oligofructose-enriched inulin	• Synbiotic supplementation reversed azoxymethane-induced downregulation of natural cell-like cytotoxicity in Peyer's patches as compared with control animals. • Synbiotic stimulated production of IL-10 in Peyer's patches of rats stimulated with azoxymethane, and in mesenterial lymph nodes of rats not stimulated with azoxymethane. • Synbiotics suppressed the production of IFN-γ in Peyer's patches. • Synbiotic suppressed the proliferative responsiveness of lymphocytes in azoxymethane-induced rats. • Synbiotic primarily modulated immune functions in Peyer's patches, thereby reducing the number of colon tumour cells.	Roller et al. (2004)
	In vivo: Azoxymethane-induced experimental colon cancer in male F344 rats	31 weeks	*B. lactis*, *L. rhamnosus*, oligofructose-enriched inulin	• Rats treated with synbiotic had remarkably lower number of tumours as compared to control rats. • Elevated level of caecal	Femia et al. (2002)

(continued)

Table 3.1 (continued)

Type of cancer	Type of study	Duration of supplementation	Content	Key findings	Reference
				short-chain fatty acids was observed in rats treated with synbiotic. • Lower level of colonic proliferation was reported in the group taking synbiotics in contrast to control. • iNOS and glutathione *S*-transferase expressions were suppressed in tumours of rats in the synbiotic group. • Reversed the elevation in the production and expression of COX-2 in the tumours of rats in the synbiotic group.	
	Randomized controlled clinical trial: Colorectal cancer patients undergoing radical colorectal resection	3 days preoperative	*B. longum*, *L. acidophilus*, *E. faecalis* (No prebiotic)	• Remarkable elevation in *Bifidobacterium* with remarkable suppression in *Escherichia* on postoperative days 3–5 in patients receiving probiotics. • Lower levels of D-lactic acids, endotoxins, CRP, and serum IL-6 in patients receiving probiotics, but with greater levels of serum IgA and IgG as compared with placebo group. • Overall incidences of	Zhang et al. (2012)

				postoperative infectious complications were 10.0% in the probiotic group in contrast to 33.3% in the placebo group.	
Hepatic cancer	Randomized controlled clinical trial: Hepatic cancer patients undergoing hepatectomy	14 days preoperative, 11 days postoperative	*B. breve*, *L. casei*, galacto-oligosaccharides	• Less profound postoperative decrease in the activity of serum diamine oxidase was observed in the synbiotic treated group, indicating restoration of intestinal mucosal integrity. • Patients in synbiotic treated group had decreased serum IL-6 and CRP concentrations. • Postoperative infectious complications did not occur in any patients treated with synbiotics in contrast to 17.2% of patients in control.	Usami et al. (2011)
Lung cancer	Randomized controlled clinical trial: Lung cancer patients undergoing chemotherapy	3 weeks	*C. butyricum* (No prebiotic)	• A lower incidence of chemotherapy-induced diarrhoea was reported in the group taking probiotics in contrast to control. • Probiotic decreased the ratios of neutrophil to lymphocyte ratio and platelet to lymphocyte, while the ratio of lymphocyte to monocyte	Tian et al. (2019)

(continued)

Table 3.1 (continued)

Type of cancer	Type of study	Duration of supplementation	Content	Key findings	Reference
				was increased. • A prominent increase in beneficial flora such as *Lactobacillus* and *Clostridium* was observed in the probiotic group. • Probiotic reduced systemic inflammatory response and promoted homeostasis.	
Oesophageal cancer	Randomized controlled clinical trial: Advanced oesophageal cancer patients receiving neoadjuvant chemotherapy	2 days prior to chemotherapy and 6 weeks throughout chemotherapy	*L. casei*, *B. breve*, galacto-oligosaccharides	• The numbers of beneficial bacterial species *Bifidobacterium* and *Lactobacillus* were remarkably larger in the group taking synbiotics in contrast to control. • The numbers of harmful bacterial species *C. difficile, Staphylococcus*, and *Pseudomonas* were remarkably smaller in the group taking synbiotics in contrast to control. • Higher levels of acetic acid and propionic acid were observed in the group taking	Motoori et al. (2017)

				synbiotics in contrast to control. • Remarkably lowered occurrence of diarrhoea and severe lymphopenia was observed in the synbiotic group as compared with control. • Fewer occurrence of febrile neutropenia in the synbiotics group in contrast to control.	
	Randomized controlled clinical trial: Advanced oesophageal cancer patients receiving neoadjuvant chemotherapy	3 days prior to chemotherapy and throughout chemotherapy	*L. casei*, *B. breve*, galacto-oligosaccharides	• Incidence of febrile neutropenia was reduced in the group taking synbiotics. • The numbers of *Bifidobacterium*, *Lacticaseibacillus*, and *Enterobacteriaceae* were significantly higher post-chemotherapy in patients receiving synbiotics. • Concentration of acetic acid was remarkably higher in the synbiotic group, leading to reduced severity of diarrhoea and febrile neutropenia. • Synbiotics combined with enteral nutrition was superior to prophylactic antibiotics as supportive therapy.	Motoori et al. (2022)

(continued)

Table 3.1 (continued)

Type of cancer	Type of study	Duration of supplementation	Content	Key findings	Reference
	Randomized controlled clinical trial: Oesophageal cancer patients undergoing esophagectomy	21 days postoperative	*L. casei*, *B. breve*, galacto-oligosaccharides	• The postoperative counts of beneficial microorganisms such as *Bifidobacterium* and *Lactobacillus*, and harmful microorganisms such as *Enterobacteriaceae*, *Pseudomonas*, and *Enterococcus* were remarkably higher and lower, respectively, in patients receiving synbiotics in contrast to control. • Concentration of acetic acid and total organic acid were larger in patients receiving synbiotics in contrast to control. • Intestinal pH was reduced in the group taking synbiotics in contrast to control. • A lower rate of infection at 10% was reported in the group taking synbiotics in contrast to 29.4% in the control group. • Systemic inflammatory response syndrome had a	Tanaka et al. (2012)

				shorter duration in the synbiotic group as compared with control group. • Overall incidence of enteral nutrition interruption due to abdominal symptoms was lower at 6.7% in the synbiotic group in contrast to 29.4% in control.	
	Randomized controlled clinical trial: Oesophageal cancer patients undergoing esophagectomy	7 days preoperative, 14 days postoperative	*L. casei*, *B. breve*, galacto-oligosaccharides	• Significantly higher neutrophil count was observed in control on postoperative days 1, 2, and 7 as compared to the synbiotic group. • More prevalent detection of bacteraemia the day after operation in control as compared with the synbiotic group. • More microorganisms were detected in mesenteric lymph node in control in contrast to the synbiotic group. • Administration of synbiotics lowered the presence of bacteria in mesenteric lymph nodes and blood, thereby reducing inflammatory response	Yokoyama et al. (2014)

(continued)

Table 3.1 (continued)

Type of cancer	Type of study	Duration of supplementation	Content	Key findings	Reference
	Randomized controlled clinical trial: Patients requiring neoadjuvant chemotherapy for oesophageal cancer	7 days prior to neoadjuvant chemotherapy	*L. casei*, *B. breve*, galacto-oligosaccharides	• The presence of bacteria was only observed in 2 of 100 blood samples in the synbiotic group in contrast to 16 of 101 samples in control. • The presence of bacteria was not detected in any of the mesenteric lymph node samples in the synbiotic group in contrast to 12 of 34 samples in control. • Synbiotic increased the concentration of faecal acetic acid thereby lowering faecal pH. • Gastrointestinal toxicity rate was reduced in the group taking synbiotics in contrast to control. • Synbiotics prevented bacterial translocation and subsequent bacteraemia that may be induced by neoadjuvant chemotherapy.	Fukaya et al. (2021)
Periampullary neoplasm	Double-blind, randomized controlled clinical trial: Patients undergoing surgery for periampullary neoplasms	14 days	*L. rhamnosus*, *L. acidophilus*, *B. bifidum*, *L. casei*, fructo-oligosaccharides	• Remarkably lower incidences of postoperative infection at 26.1% was reported in patients receiving synbiotics in contrast to	Sommacal et al. (2015)

				69.6% in the control group. • Antibiotic therapy mean duration was shorter at 9 days in patients receiving synbiotics in contrast to 15 days in the control group. • Lower rate of non-infectious complications (6 of 23 patients) in patients receiving synbiotics in contrast to control (14 of 23 patients). • Mean hospital stay length was shorter at 12 ± 5 days in patients receiving synbiotics in contrast to 23 ± 14 days in control. • Six deaths were reported in control while no deaths were reported in patients receiving synbiotics. • Synbiotics reduced postoperative mortality and incidences of complications.	
Prostate cancer	Double-blind, randomized controlled clinical trial: Prostate cancer patients undergoing radiotherapy	1-week pre-radiotherapy and 4 weeks post-radiotherapy	*L. reuteri*, soluble fibre	• Synbiotics reduced symptoms of proctitis. • Synbiotics improved the quality of life in radiation-induced acute proctitis in prostate cancer patients undergoing radiotherapy.	Nascimento et al. (2014)

complications. As such, future research should focus on employing synbiotics as a cancer therapy that could target and modulate tumour cells directly. The underlying mechanisms of synbiotics in modulating the gut microbiome to exert an anticancer effect should also be thoroughly investigated, which could enable the development of effective strategies for early detection and treatment of tumours by cancer researchers. In a nutshell, with rapid advancement in medical technology and deeper insights into oncology and human microbiome research, it is believed that synbiotics can be translated into a novel cancer prevention and treatment strategy upon successful execution of clinical trials in the near future.

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Correlation Between Reactive Oxygen Species and Synbiotics for Effective Treatment of Cancer

4

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4.1 Introduction

Cancer is the third most prevalent related cause of death in the world, according to the National Cancer Institute. The World Health Organization estimates that around 27 million new instances of cancer will be diagnosed worldwide by 2030, resulting in the death of 17 million people. In the future, the World Health Organization predicts that approximately 75 million people would be afflicted by cancer in the United States. Furthermore, in addition to genetics and lifestyle factors, the etiology of colorectal cancer is complex and includes a combination of environmental and genetic factors that may cause changes in the intestinal microenvironment that result in carcinogenesis. Recent years have seen an increase in the interest in dietary methods for the prevention of cancer particularly when it comes to dietary restrictions and modifications. There have been numerous studies in clinical and epidemiological settings that have demonstrated the therapeutic effects of various nutrients and food constituents, such as calcium and selenium. Pericleous et al. (2013) suggested the roles of nutrition in the development of cancer, diet with high vitamin D content, n-3 fatty acids and digestible fibre may protect against colon cancer (Pericleous et al. 2013; Wu et al. 2018; Yang and Yu 2018). Extrinsic (i.e., environmental) factors such as infectious agents, antibiotic administration,

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high-fat diets, red meat consumption, and a lack of fiber intake, on the other hand, have a considerable impact on cancer risk (varying from 10% to 30%) (Wu et al. 2016).

Furthermore, all of these components have been shown to have an effect on the gut microbiota and cause dysbiosis, which is defined as disturbances of commensal communities that can result in a lack of immunological education in the host and the development of immune-mediated illnesses as a result of this lack of immunological education. The term dysbiosis refers to a state of affairs in which beneficial microorganisms have been eliminated, pathobionts or potentially hazardous microorganisms have multiplied, and total microbiological diversity has been eliminated (Almeida et al. 2018). A new study has discovered that patients with colorectal cancer have all three types of dysbiosis, according to the findings. The production of helpful metabolites by a healthy gut microbiota is one of the ways in which they can exert their anticancer benefits. One approach is by functioning as vitamins and as a source of energy. In addition to having antioxidant and anti-inflammatory effects, these beneficial metabolites can also control intestinal barrier function and serve as a source of energy. There is evidence that the gut microbiota of cancer patients can directly promote tumorigenesis; for example, fecal samples from cancer patients have been reported to produce intestinal carcinogenesis in germ-free mice and conventional mice when given to animals using a gavage procedure (Wong et al. 2017).

While prebiotics (nondigestible food ingredients that promote the growth of beneficial resident bacteria) are linked to the development of precancerous colonic lesions, the consumption of probiotics (living organisms believed to have a beneficial effect on health) is linked to the development of precancerous colonic lesions (Pool-Zobel 2005). Precarcinogenic food compounds can bind to synbiotics, reducing the production of bacterial enzymes that hydrolyze them, and reducing intestinal inflammation. Clinical trials have shown that synbiotics (a combination of probiotics and prebiotics) has a synergistic protective effect against the development and progression of cancer. Bacteria colonizing the intestine has been a hot topic recently, with many researchers questioning whether or not they have a role in cancer formation. When people with healthy gut microbiota compare their samples to those from people with colorectal cancer, it has been found that dysbiosis is a risk factor. Cancer cannot be linked empirically to a single pathogen, hence it is more likely caused by a host's interaction with an unbalanced gut microbiota, a condition known as dysbiosis, than the other way around (Bakhtiar et al. 2013).

It is estimated that the human intestinal microbiota contains trillions of bacteria, all of which colonize and disperse themselves at specific places in the body, where they form complex communities. Those that appear in the colon are the most numerous (approximately 10 microorganisms/g of intestinal content). The gastrointestinal tract contains helpful bacteria that are both locally and systemically useful in that they regulate intestinal homeostasis and neuromuscular function of the gastrointestinal tract (Sobhani et al. 2011). A possible mechanism by which the intestinal microbiota can interfere with the carcinogenic process is because of its potential to stimulate host immune responses, alter tumor cell metabolism, and govern cell death

and proliferation, among other things. Absorption and separation of bile acids are made easier as a result of this process, which has been found to enhance oxidative stress in experimental animals, damage DNA, and contribute to mitochondrial membrane instability. Probiotics are the most commonly utilized method of changing the intestinal flora. The microbiota and the host benefit from probiotics.

According to the American Cancer Society, research has shown that nondigestible food components known as prebiotics can help prevent cancer by encouraging the growth of good bacteria and the proper functioning of the colonic microbiota. When probiotic bacteria multiply, short-chain fatty acids (SCFAs) are produced in varying amounts, which is why they are beneficial. Probiotics and prebiotics alone may not be as effective in preventing cancer as synbiotics, which combine the two. Researchers found that combining a starch-resistant prebiotic with the probiotic Bifidobacterium lactis dramatically increased the mortality of colon cells in rats exposed to a carcinogenic toxin. To lessen the danger of side effects or to treat a wide range of disorders, Tárraga López et al. (2014) claim that natural alternatives to synthetic medications are becoming increasingly popular.

Probiotics and synbiotics may be useful in reducing cancer risk, particularly in the case of colorectal cancer, which has a high death rate around the world and is an aggressive tumor (Cruz et al. 2020). Disruption of the gut microbiota can lead to poor host health and the start of disease, which is why it is so important for human body homeostasis. Researchers are excited about the potential of probiotics in the treatment of colorectal cancer, and these microbes have shown their ability to aid in the process. The specific impacts of biological responses connected to colorectal carcinogenesis, particularly those relating to intestinal microbiota composition and changes caused by colorectal cancer, are not well understood. Microbes associated with cancer patients' mucosa differ dramatically from those of healthy individuals. Among other things, the microbiota of patients with colorectal cancer tends to become more diverse as the disease advances (Cruz et al. 2020). To find out if probiotics and synbiotics have a place in the fight against cancer, as well as to learn more about the underlying mechanisms that contribute to the disease's progression, this review was carried out. Having a healthy gut microbiome is critical to general health.

4.2 Reactive Oxygen Species (ROS) Formation

Molecules and free radicals generated by molecular oxygen are known as "reactive oxygen species" (ROS). The production of oxygen-based radicals is a problem that confronts all aerobic creatures. Aerobic respiration, mitochondrial electron transport, or metal-catalyzed oxidation can all produce these compounds, which can lead to oxidative stress. All aerobic creatures require this process in order to survive. While ROS production and ROS removal are now balanced, there are a wide range of defense mechanisms in place to meet this need. Oxidative stress is a condition in which the body's oxidative capacity is being strained (Bergqvist et al. 2020). Cells use a range of defensive mechanisms to fight ROS. Superoxide dismutase (SOD) can

act as a catalyst in a process to produce H_2O_2 and oxygen from two super oxide anions ($O_2^{-\bullet}$)

4.2.1 Types of ROS

Oxygen, oxygen radical$^{\bullet}$, hydrogen peroxide, and hydrogen peroxide are the most frequent ROS. When in its ground state, oxygen has two unpaired electrons with parallel spin, making it a paramagnetic molecule that is unlikely to interact with organic molecules until activated. Oxygen molecule can be activated using any of the following two approaches: Step-by-step monovalent reduction and absorbing enough energy to reverse an unpaired electron's spin are two ways to do this. There are two ways to make O_2: the first is to add oxygen to the air, and the second is to decrease it to O_2, H_2O_2, and OH (Bergqvist et al. 2020). The chloroplast, mitochondria, and peroxisomes are the only plant cell compartments where reactive oxygen species (ROS) can be produced continuously during aerobic metabolism. Moreover, a new study has shed light on the apoplast's importance as a generator of reactive oxygen species, as recently discovered data indicated (ROS). As long as there are suitable conditions, ROS production continues at a steady rate. To protect themselves from injury, they are scavenged by a variety of antioxidative systems (Sredoja Tisma et al. 2021).

Oxygen in the biradical state has a spin that is parallel to the spin of the atom's nuclei. Singlet states are formed when enough energy is absorbed to reverse one of the unpaired electrons' spin, creating an electron pair with the opposite spin. By participating in processes involving the simultaneous transfer of two electrons, O_2 is able to break past the spin barrier (divalent reduction). ROS are primarily produced in plants by the mitochondria, which are the most abundant organelle in plants, followed by chloroplasts and peroxisomes. The endoplasmic reticulum, cell membrane, cell wall, and apoplast are only a few examples of secondary sites. Under diverse environmental stress circumstances such as salt, drought, cold, heavy metals, UV irradiation, and so on, ROS family members play a vital dual function in maintaining normal cellular homeostasis.

Oxidative damage can occur as a result of their role as secondary messengers in a number of physiological processes (Yang et al. 2021). The breakdown of biomolecules including pigments, proteins, lipids, carbohydrates, and DNA occurs as a result of cellular damage, and this results in the death of the plant's cellular structure and function. Researchers found that plants have evolved a robust antioxidant apparatus with two arms, including SOD (superoxide dismutase), DHR (dehydro-ascorbate reductase) works together to scavenge ROS (Yang et al. 2021). The delicate equilibrium between ROS formation and ROS scavenging is disrupted by several stress conditions, such as salt, aridity, high temperatures, heavy metals, and pathogen infection, among others. The severity and duration of stress episodes, as well as the plants' ability to quickly react to fluctuating energy balances, are all important considerations for plants' survival (Wang et al. 2017). Residual oxygen

species are believed to be produced in plants using only 1–2% of the oxygen available to the ROS.

ROS were formerly thought to be primarily produced by mitochondrial metabolism. This is contrary to conventional opinion, as research has shown that NADPH-oxidase enzymes create a significant amount of ROS in people. There are many different reduction stages toxic reactive oxygen species (ROS) might go through before they affect healthy cells. To ensure the survival of all cells in the body, the detoxification of reactive oxygen species (ROS) is essential. Numerous defense systems had to be developed in order for live organisms to survive in the oxygen-rich cellular environment. The purpose of these defense mechanisms is to protect against reactive oxygen species (ROS). An imbalance between ROS production and cell ability to rapidly detoxify or recover from ROS-induced damage is referred to as oxidative stress.

Oxidative stress, the outcome of an imbalance between the creation and detoxification of reactive oxygen species, leads to cellular failure (ROS). ROS causes lipid peroxidation, nucleic acid alterations, and protein aberrations in biological macromolecules. Their formation has been related to atherosclerosis (ischemic heart disease), diabetes, and the development of carcinogenesis or liver disease. In order to maintain proper cell signaling, several radical scavenging enzymes are considered to keep ROS levels within the cell below a certain threshold (Woelk and Snyder 2021). An increase in ROS synthesis can result in excessive cell signals and damage to critical components of the signaling pathway, but there is a limit to the quantity of ROS that can be created. ROS has the potential to permanently harm vital macromolecules. The –SH group in the protein-bound and non-protein thiol compounds provides a cellular reducing and protective agent against a wide spectrum of hazardous substances, including most inorganic contaminants. The initial line of defense against oxidative stress is often thiolactone.

4.3 Carcinogenesis

Carcinogenesis is the process of transforming normal cells into cancerous ones through uncontrolled proliferation and genetic abnormalities (Pu et al. 2020). Carcinogenesis is usually divided into phases. A normal cell becomes a tumor cell after irreversible changes to its DNA (deoxyribonucleic acid) in the nucleus; tumor promotion occurs when a clone of initiated cells proliferates abnormally; and tumor progression occurs when precancerous lesions become malignant lesions due to initiated cell proliferation. Cancer cells eventually acquire features that distinguish tumors from healthy tissue (Flemer et al. 2018). These include the ability to proliferate, resistance to apoptosis (programmed cell death), and angiogenesis (tumor-specific vascular growth) (dissemination through the blood or lymphatic system to distant organs). Toxic substances damage chromosomes and genetic make-up (DNA). This is especially true of several substances to which employees are often exposed. Throughout a cell's life, DNA gets attacked, but the repair processes usually heal the damage.

However, failure or suppression of essential gene repair processes can cause or exacerbate cell transformation and thus carcinogenesis, especially when environmental factors are involved. When a cell splits, its genetic material is passed on to the daughter cells (Liu et al. 2020). Unrepaired DNA lesions can cause genome-wide changes like chromosomal rearrangements or gene mutations. Oncogenes and tumor suppressor genes are required for cell growth, division, differentiation, and death. This allows for balanced cell division. Mutations in these genes promote the growth of a clone of abnormal cells. To finish the process of carcinogenesis takes years or perhaps decades.

4.3.1 Stages of Cancer and Its Management vis a vis Chemoprevention Cum Gut Microbiota

Oncogenic pathways are needed to understand cancer etiology and pathology. Gut microbiota and carcinogenesis: environmental and genetic factors (Hekmatshoar et al. 2019). The indirect bacterial process of oncogenesis is shown in the course of chronic inflammation caused by bacterial infections. The microbiota can activate the transcription factor nuclear factor-kB (NF-kB) and hence contribute in the formation of malignant tumors by producing inflammation mediators such as TNF-a and IL-1. Also, metabolites or toxin generated by bacteria can initiate bacterial oncogenesis. Previous research has connected gut microbiota to cancers such gastric cancer, colorectal cancer, and hepatocellular carcinoma (HCC).

All of these carcinogenic processes share the same trait: microbial metabolite production (Liu et al. 2020). SHP2 and PAR1/MARK interact with *H. pylori* CagA proteins to promote carcinogenesis. *Bacteroides fragilis* is an opportunistic pathogen. Colorectal cancer can be caused by toxic *B. fragilis* (ETBF), one of two *B. fragilis* subtypes (CRC). When *B. fragilis* toxin is present, decreased spermine oxidase activity causes reactive oxygen species (ROS) and indirect DNA damage. *Pasteurella multocida* toxin, CDT, and IPPPD have also been linked to cancer risk (IpgD). All of these factors may affect cellular responses, increasing the risk of cancer (Tsvetikova and Koshel 2020). A healthy gut microbiome profile is thought to be adequate for a healthy microbiota. Aiming for dysbiosis may improve the prognosis and reduce negative effects of various anticancer drugs (Fig. 4.1).

4.4 Gut Microbiota and Cancer

Recent research shows that gut microorganisms impact a host's overall health. Various metabolites and bioproducts produced by gut bacteria safeguard the host and gut homeostasis. In contrast, pathological dysbiosis may increase the number of microbiota subpopulations that produce toxins that can cause inflammation and cancer. Gut microbial interactions can alter the host's immune system and gut epithelium (Yang et al. 2019).

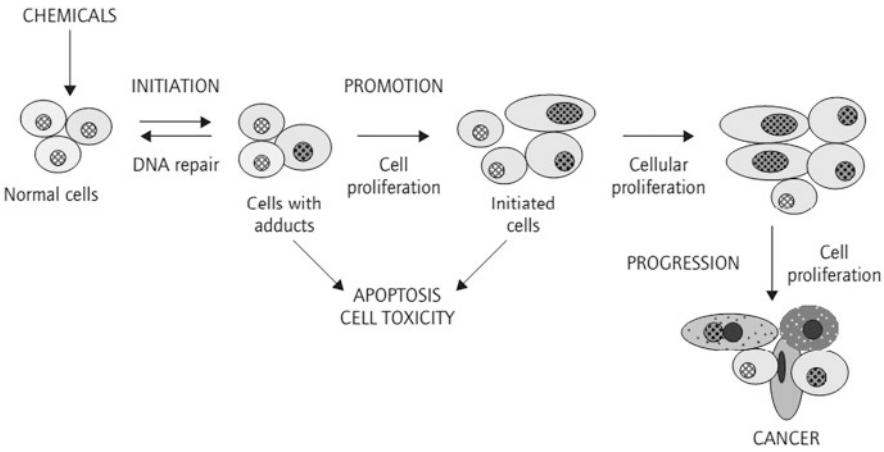

Fig. 4.1 Steps involve in carcinogenesis

Probiotics, or intestinal microorganisms, have been shown to protect against cancer growth. Probiotics are currently being examined to determine if they can help dysbiosis-prone cancer patients undergoing chemotherapy and radiotherapy. Three recent research suggest that certain gut-dwelling species may benefit anticancer treatment. Even if these treatments are effective of suppressing the progression or even killing some types of cancer cells, there remain limitations due to developed resistance and unpleasant side effects (Winkler et al. 2014).

The human epithelia contain a range of microorganisms, the most common being commensal bacteria found in the gastrointestinal tract, collectively known as the human microbiota. High-throughput sequencing has made it possible to pinpoint the gut microbiota's composition. Healthy people have the following bacterial types: non-Actinobacteria, proteobacteria, or firmicutes microorganisms. Many cancer drugs may benefit from better gut microbiota control, according to recent research linking gut microorganisms and treatment results, including reduced cytotoxic activity (Chang et al. 2020).

4.4.1 Microbiota and Chemotherapy

Chemotherapy is one of the most effective systemic cancer treatments today. Chemotherapeutic drugs that target DNA, topoisomerase, or tubulin can stop cancer cells from growing and multiplying. Despite this, there are unavoidable adverse effects due to the lack of specific chemotherapeutic targets. It was eventually discovered that gut microorganisms and cytotoxic drugs interact in two ways. Chemotherapy reduces the number and diversity of bacteria in several preclinical studies. A decrease in *Lactobacillus* and *Bifidobacterium* and an increase in *Escherichia coli* (*E. coli*) and *Staphylococcus* has been observed in clinical studies. The changed microbiota composition causes an inflammatory response and reduces

barrier function, exposing the host to pathogens (Schirmer et al. 2016). The microbiota in the stomach can affect the efficiency of chemotherapy in two ways. Many anticancer drugs that are given orally or injected into the body rely on gut microbes to become active. In CRC treatment, intravenous carboxylesterase converts CPT-11 (irinotecan) to SN-38.

The drug's active ingredient prevents DNA ligation, causing single and double strand breaks. UDP-glucuronosyl transferase then detoxifies it (UDP-transferase). It was shown that SN-38 levels in feces increased from 2% to 12% of the dose when deconjugation by gut microbiota generated beta-glucuronidases occurred. According to the research, microbes have a vital role in drug. Alternatively, gut bacteria may help synthesize chemicals that block an enzyme used in drug detoxification, causing more severe adverse effects. When rats are given 5-FU with Sorivudine, Sorivudine is transformed to bromovinyluracil, which further inhibits the 5-FU detoxifying enzyme dihydropyrimidine dehydrogenase. This is a prevalent topic in 5-FU toxicity studies. 5-FU, which aids in DNA replication, can cause diarrhea and even decrease of leukocyte and platelet counts if used long term and in high doses (Klaassen and Cui 2015). This conversion occurred solely within the human body and was proved by experiment to be due to gut microbiota species, revealing the crucial role gut microorganisms play in chemotherapy-induced harm. The gut microbiota may increase the toxicity of chemotherapy treatments while simultaneously helping them fight cancer. Increasing the expression of reactive oxygen species-producing enzymes (ROS) (Woelk and Snyder 2021). Defensively, the gut microbiome may help chemotherapy work better and less harmful. Gene silencing can increase the expression of ROS-generating enzymes. ROS can damage DNA and trigger apoptosis in tumor cells when employed as a chemotherapeutic. Less expression of Nox1 and Cybb genes coding for NADPH oxidase 2 genes in germ-free mice or mice treated with an antibiotic cocktail (ABX) may minimize anticancer effects (Nox2). Enzyme compartment-specific superoxide dismutases (SOD) can produce H_2O_2, causing DNA damage in tumor cells and necrosis (Fig. 4.2) (Chang et al. 2020).

4.5 Synergy of Probiotics and Prebiotics and Mode of Action

In silico evaluation and metagenomics have been used to study a quiet of useful and important diverse group of microbes that reside in the human gut microbiota. Currently, the study on gut microbiota is continually gaining attention as their role in protecting against diseases are significant. Prebiotics and probiotics are two important food components that are needed in the human gastrointestinal tract. The human gastrointestinal track can house viable microbes which when ingested can help prevent against some pathological conditions, such organisms are widely regarded as probiotics. These microbes found in some foods, thereby interacting with the indigenous colon organisms limiting the concentration of the pathogenic ones (Sanders et al. 2019). In other hand, prebiotics are compounds or structure which supports the growth of probiotics.

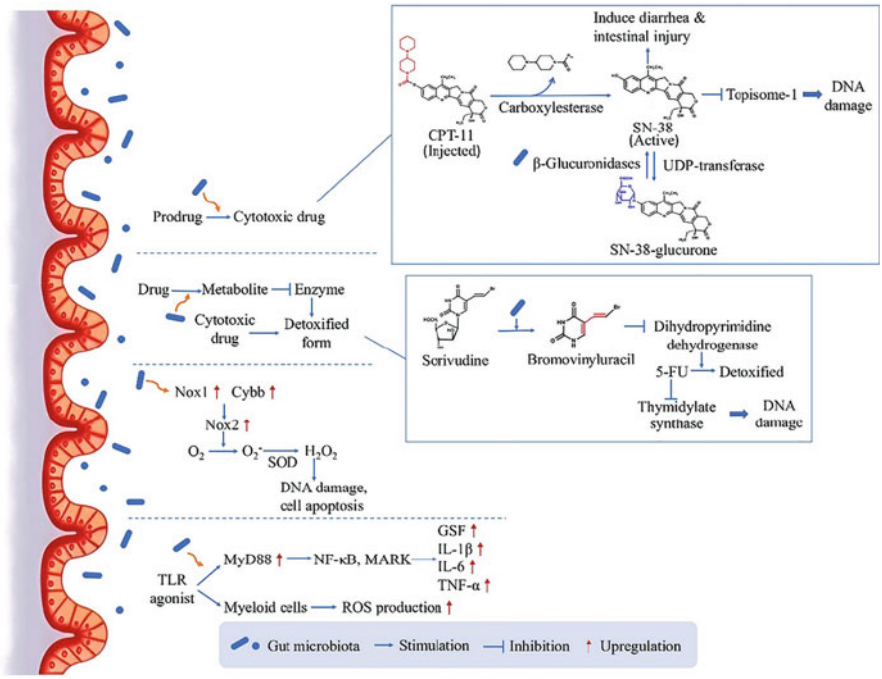

Fig. 4.2 Drug pharmacokinetics and ROS generation are directly influenced by bacteria. The gut microbiota can directly influence medication conversion and gene transcription, resulting in either an increased therapeutic impact or an increased adverse effect (Li et al. 2021)

Consumption of some foods which includes butter, yogurt, and milk and other fermented foods have been studied to be rich in probiotics, the discovery of such food is describe through a phenomenon called probiosis. Probiosis is a term used to describe the correct consumption of fermented products with cultures of beneficiary microbes (Mtasher et al. 2018). Fermented foods have long taken as a nutritional food, but off recent their importance in clinical settings. Obstruction of the normal floral of the gut is accompanied by the overgrowth of pathogenic microbes leading to significant loss diversity of important gut microbes and as such inflammatory response is built up by the host causing the disease conditions (Molehin et al. 2022). Prebiotics exclusively enhances the proliferation of beneficial bacteria in the gastrointestinal track. Live organisms such as probiotics confers a health importance on the host when taken appropriately in the right food components. Probiotics mode of action is not fully outlined and understood, but their survivals in the intestine brings about stability of the intestinal ecosystem. The criteria for optimal activities of probiotics is believed to be depending on the number or population of the viable cultures of medical significance and the bio-efficiency of the prebiotics which helps stimulate their growth (Patel et al. 2015).

The probiotic community may include on or more microbial strains which may include *Bacillus* spp., *Enterococcus* spp., *Lactobacillus* spp., *Pediococcus* spp., and *Streptococcus* spp. Since microorganisms are fully involved in both probiotics and prebiotics, the assessment of such strains for safety is very important weather which strains are being optimized to maximized it health-related positive effect. However, the mechanism of actions of probiotics is not fully understood, but it has been reported that they are capable of surviving the harsh environment of the alimentary canal while offering a beneficiary effect on their host (Anadón et al. 2014). The presence of these beneficiary microorganisms has been studied to improve digestion and enhancing metabolic and immunological processes.

Therefore, properties of probiotics are to enhanced health and increase productivity of animals. Microorganisms with probiotic capacities are well structured to adhere to epithelial cells subsequently blocking or hindering the binding of their receptors to the epithelial cells. The blockage by probiotic bacteria is as a result of competition and production of anti-adhesive effect leading to activation of mucin (a complex glycoprotein mixture). Many scientific studies have shown how different lactobacilli glycoprotein promotes their subsequent binding to the mucosal membrane mediating their surface attachments layer (González-Rodríguez et al. 2012).

There are many groups of organisms that have been explored and used as probiotics. It is important to note that many of these genera have similar biochemical, physical, and metabolic characteristics. The Lactobacillus group are the most profound of all the probiotics, they are Gram-positive rod-shaped microorganisms possessing the ability to produce an organic acid called lactic acid. These group of organisms are regarded as friendly microbes as their colonization proved to be beneficiary and nutritional (Table 4.1).

Probiotic bacterium plays very important role in several health challenges and performance. Some of the importance include their therapeutic effect, well-structured microbial concentration in the intestine, and other aiding several immunomodulatory and metabolic responses (Anandharaj et al. 2014). Despite several colonization of microbes in the colon, there are several idea requirements for being a probiotic bacterium. Such requirement includes the following (Behnsen et al. 2013)

1. For an organism to be regarded as probiotic, they should give a positive effect on the host gastrointestinal tract.
2. Such organisms must strive in the presence of acidic nature of the stomach while also resisting the antimicrobial effect of bile.
3. The binding to their host mucosal surfaces must be fast and firm without easy of obstructions.
4. They must be able to propagate themselves easily and faster.
5. They must be capable to exclude pathogenic invasion by blocking the adherence of the pathogens with the epithelial tissues.
6. Even when heavily populated, they must be safe, noninvasive, nonpathogenic, noncancerous to their host.

Table 4.1 List of commonly used microbes as probiotics

Genus	Species
Lactobacillus spp.	*acidophilus* *plantarum* *rhamnosus* *paracasei* *fermentum* *reuteri* *johnsonii* *brevis* *casei* *lactis* *delbrueckii gasseri*
Bifidobacterium spp.	*breve* *infantis* *longum* *bifidum* *thermophilum* *adolescentis* *animalis* *lactis*
Bacillus spp.	*coagulans*
Streptococcus spp.	*thermophilus*
Enterococcus spp.	*faecium*
Saccharomyces spp.	*cerevisiae*

7. With other nonpathogenic bacteria, they must be able to form a conglomerate of balance normal flora.
8. They must be durable and have capabilities to withstand commercial processing.

4.6 Antioxidation Properties of Probiotics

Many scientific reports have confirmed that probiotics is capable of lowering lower the onset and severity of diarrhea, aside from that, the presence of probiotics in a compactible host help regulate both the active and passive immunity while preventing tumor cells proliferations and decreasing metabolism of ammonium containing foods and pro-cancerogenic enzymes in the stomach (Mishra et al. 2015). It has been studied that probiotic has resolved various metabolic processes such as diabetes and obesity through modifying intestinal microorganisms (Rad et al. 2016). Oxygen is regarded as an important factor negatively influencing the survival of anaerobic organism of any kind. The oxygenic environment is thought to stimulate the production of toxic end product stimulated by oxygen.

Oxidative stress is biochemically described as a condition which disturbs the prooxidant-antioxidant balance within the cell. As a result, DNA hydroxylation, denaturation of protein, peroxidation of lipid, and untimely programmed cell death are all being initiated following the oxidative stress. As such, oxidative stress increases the intracellular oxygen expenditure and radicals leading to reactive

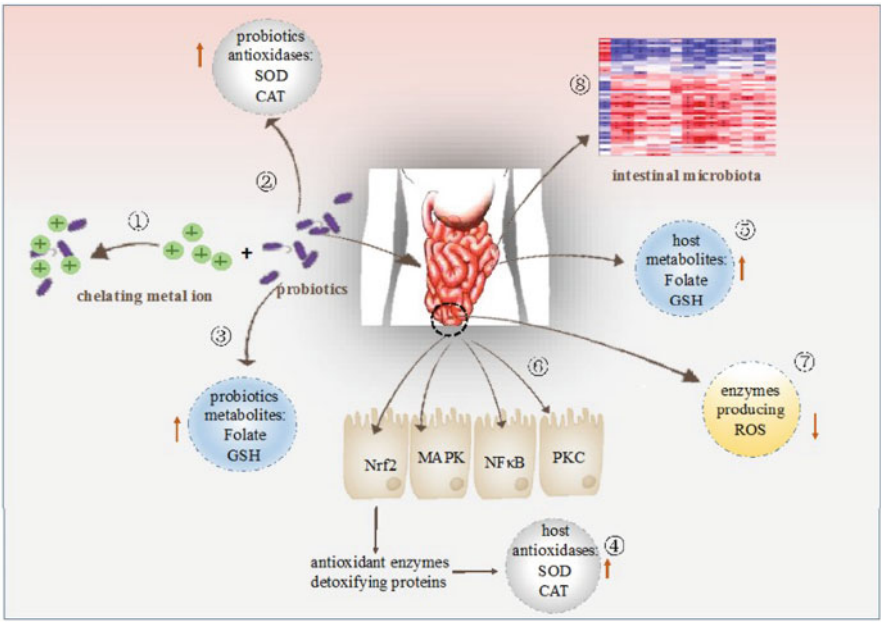

Fig. 4.3 Mechanism of antioxidative modulation by probiotics (Wang et al. 2017)

oxygen species (ROS), superoxide anion radicals, and hydrogen peroxide (Schieber and Chandel 2014). Most living organisms are programmed to defend antioxidant imbalance many of which are enzymatic in nature such as superoxide dismutase, and some are non-enzymatic based such as Vitamin C and E, all which are not enough for antioxidative defense (Mishra et al. 2015).

Most studies on probiotics and prebiotics have mainly focused their anti-diarrhea potentials, immune-stimulating factors, immunomodulation, and their ability to reduce unpleasant metabolites in the body while less focusing on their anticancer and antioxidative potentials (Mishra et al. 2015). The antioxidative potential of probiotics is currently gaining focus. Recently, cultures of *Bifidobacterium animalis* were observed to scavenge superoxide anion and hydroxyl radicals in vitro while encouraging antioxidase activities. Oxidative stress of type 2 diabetic patients has been studied to recede following the ingestion of probiotics (Fig. 4.3) (Wang et al. 2017).

Probiotic organisms scavenge free radical through various means. Probiotic microbes can act as chelators such as penicillamine and ethylene diamine tetra-acetic acid (EDTA) which enables to capture metallic ions subsequently preventing them from further oxidation. Cellular apparatus responsible for these chelating potentials are not well understood (Wang et al. 2017). Since probiotic microbes are living entities themselves, they possess their own antioxidative system. One of its well-studied antioxidative systems are the antioxidant enzymatic apparatus observed are superoxide dismutases (SOD).

Furthermore, probiotics can stimulate antioxidases from the host antioxidative apparatus (Wang et al. 2017). Probiotics can also serve as antioxidant metabolite through production of various metabolite such as folate, butyrate, and glutathione. It is important to note that folate is a very important vitamin needed during DNA replication, optimization of DNA repair mechanisms, and DNA methylation (Wang et al. 2017).

4.7 Effective Management of Cancer Through Symbiotics

There are investigations regarding the combine use of probiotics and prebiotics in the management of cancer. Many anticancer activities of probiotics such as *Lactobacillus* spp., *Bifidobacteria* and many others have been studied to have antimutagenic potentials due to the fact that they are capable of metabolizing and inactivating mutagenic compounds. In other studies, the anticancer effect of probiotics is also strengthened by their ability to inhibit procarcinogen which transforms to active carcinogens, reduction and inactivation of mutagenic compounds, and the subsequent reinforcement and optimization of functions for the immune system (Fig. 4.4) (Soccol et al. 2010).

The antitumor potentials of probiotics are based on the following functions which are: upregulation of metabolic activity of the microbes in the intestine, modification of microbial population in the intestine, production of short chained fatty acids as well as conjugated linoleic acids both which have anticancer effect, inhibition of abnormal cell proliferation coupled with immunomodulation potentials (Śliżewska et al. 2020). Imbalance in the population and varieties of microbes found in the intestine may give room for pathogenic organisms leading to dysbiosis which is termed as excess of pathogenic microbe invasions. When these conditions are not properly curtailed, they cause severe inflammation in the system which could lead to

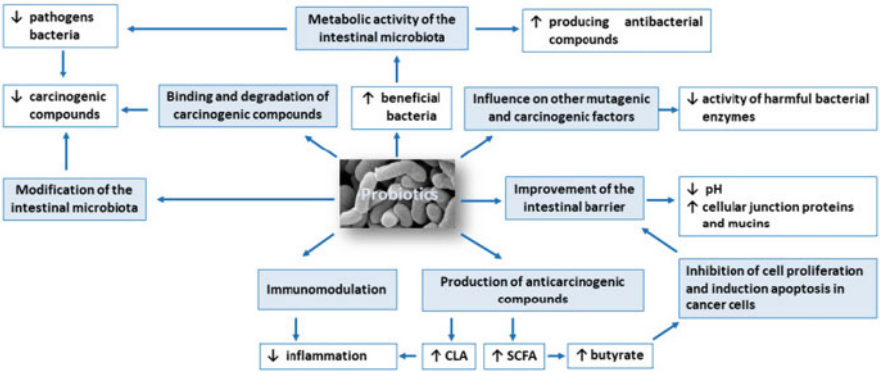

Fig. 4.4 Flowchart showing possible mode of actions of probiotics on colon cancer (Śliżewska et al. 2020)

accumulation of carcinogenic compound thus increasing the likelihood of colon cancer (Dos Reis et al. 2017).

Probiotic microbes compete with their pathogenic counterpart restricting their attachment with mucosal membranes through the development of complex and quorum sensing triggered structured called biofilm (Ohland and Mac Naughton 2010). Biofilms are formed through complex interaction within diverse genetic population where individual microbes contribute their extracellular compound by specific command called quorum sensing. Such extracellular substance includes protein, capsules, polysaccharides, and phospholipids among many others. The probiotics presence meaningfully reduces the menace of postoperative difficulties which could be anastomotic leakage, mechanical ventilation, and infections (Śliżewska et al. 2020).

In some cases, nonpathogenic microbes may covert some found components into carcinogenic compounds. For example, the ability of some microbes to produce enzymes such as nitrate reductase, azoreductase, and beta-glucosidase all which have been studied to be capable of bio-transforming heterocyclic and polycyclic aromatic compounds including bile acids into ammonia, phenols, synthetic aglycones, cresols, and N-nitroso compound all which are potential hazards to the biological system. In more severe cases, such enzymes can covert these compounds into active carcinogens (Zhu et al. 2013).

Probiotic microbes can challenge this bio-transformation by changing the microbial metabolism through modulating the actions of the enzyme concerned. *E. coli*, *Clostridium* spp., and other related microbes have higher enzyme activities required for synthesis of carcinogenic compounds, probiotic bacteria are capable of reducing the pathogenic population of such bacteria and consequently decreases the initiation of carcinogenesis (Śliżewska et al. 2020). Lactic acid bacteria (LAB) control the population of other microbes in their immediate environment by producing organic acids. Organic acids are considered as one of the major acids that helps inhibit the propagation of pathogenic microbes. The most important compounds produced by LAB are the lactic acids, acetic acids hydrogen peroxide and bacteriocin all which can inhibit the growth of pathogens with high selective toxicity (Śliżewska et al. 2020).

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Synbiotics in Colon Cancer

5

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5.1 Introduction

It's no secret that cancer is a major cause of mortality in the globe. There are various types of cancer identified. Colon cancer (CCa) is the most well-understood multistep malignancy in molecular genetics. Intestinal mucosal neoplastic polyps are the first signs of carcinogenesis. A polyp's histology is critical in determining whether or not it is cancerous. Histological classifications such as hyperplastic and adenomatous are both common. More glandular cells with less mucus but no hyperchromatic or stratification are seen in hyperplastic polyps histologically (Tsai and Lu 1995). It

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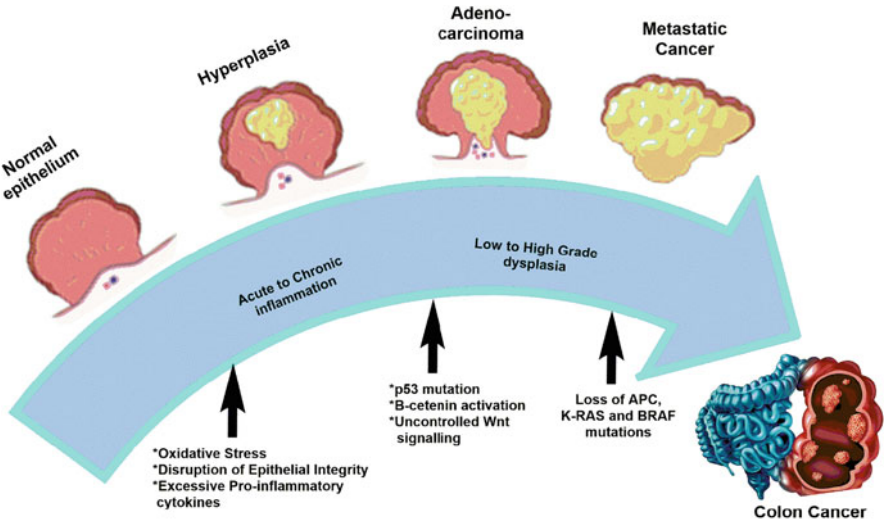

Fig. 5.1 Stages for the formation of colon cancer

is normal for adenomatous nuclei to be large, and tightly packed in a palisading pattern. Viciform villi create a frond in the villus adenomas, which are distinct from tubular adenomas, which are formed of branching tubules. The generalized lifecycle of the formation of colon cancer has been denoted in Fig. 5.1.

Adenomas, which are often observed in colon cancer patients, are thought to be the most common cause of the disease (adenoma-to-carcinoma sequence). To begin, synchronous adenomas are often seen in surgical specimens from patients undergoing treatment for colon cancer. Second, the greater the number of adenomatous polyps identified in the colon, the greater the risk of getting colon cancer (Heald and Bussey 1975). The presence of adenomatous tissue near malignant tissue is a third criterion to consider (Cappell 2005). Colon cancer is inevitable if a patient with familial adenomatous polyposis (FAP) and hundreds or thousands of adenomatous colonic polyps does not get a colectomy (McConnell 1976). When adenomatous polyps bigger than 1 cm were found in individuals who did not have colonoscopic polypectomy, colon cancer rose from 1% to 1.5% every year (Stryker et al. 1987).

However, despite the fact that most hyperplastic polyps seem to have little or no link to colon cancer, some polyps do have been linked to it (Winawer et al. 2006). More than 20 polyps in the colon, right-colon hyperplastic polyps greater than 1 cm in diameter, adenomas inside the polyp, and creating the chain of these polyps and thereby increasing the risk (Jass 2004). There appears to be a relationship between hyperplastic polyps and colon cancer in the recently classed (sessile) serrated adenoma (Higuchi and Jass 2004). The aberrant crypt epithelium growth and nuclear atypia distinguish this kind of hyperplastic polyp from others (Chlumská et al. 2006). In one investigation, polyp specimens formerly hyperplastic were substituted with serine tumors, which exhibited serrated edges (Torlakovic et al. 2003).

Serrated adenomas differ from other adenomas because colon cancer seems to grow differently. Unlike typical adenomas, these tumors include more BRAF mutations and DNA methylation, but they lack APC gene mutations (Spring et al. 2006). Approximately one-fifth of all instances of spontaneous colon cancer have a serrated adenoma, a marker of colorectal carcinoma with significant microsatellite instability at an early stage (Thibodeau et al. 1993). DNA methylation is common in serrated adenomas and microsatellite instability-high (MSI-H) colon cancer, while APC and K-ras mutations are missing (Huang et al. 1996). If there is no mutation in the DNA, DNA methylation may deactivate DNA mismatch repair genes, such as the hMLH1 gene, causing microsatellite instability (Kambara et al. 2004). A serrated adenoma is still a mystery about what precise genetic mutations produce it.

Serrated adenomas have greater probability of cancer development (Jass 2004). Firstly, it has been shown that serrated adenomas share genetic changes with previously described spontaneous MSI-H malignancies. Second, serrated adenomas in areas of severe dysplasia suggest that the dysplasia arose from this precursor lesion (Longacre and Fenoglio-Preiser 1990). Serrated adenomas and colon cancer are prevalent in patients with hyperplastic polyposis, a condition characterized by at least 30 hyperplastic polyps dispersed throughout the colon (or at least five hyperplastic polyps within 1 cm of the sigmoid colon) (Jeevaratnam et al. 1996). MSI-H colorectal tumors were found in the same area of the proximal colon where hyperplastic polyps were previously diagnosed by colonoscopy with pathologic evaluation of polyp tissue; all previously excised polyps were reclassified as serrated adenomas (Goldstein et al. 2003). The remaining traditional hyperplastic polyps are expected to provide a minimal risk for colon cancer due to the separation of high-risk serrated adenomas from hyperplastic polyps in the new nomenclature.

Molecular insights gained from studying the uncommon hereditary cancer illness FAP's pathogenesis have increased our knowledge of the molecular foundation for the development of colon cancer from sporadic adenomas (McConnell 1976). There are hundreds or thousands of adenomatous polyps in the colon of people with FAP after puberty, which ultimately lead to colon cancer. According to the Mendelian model, this disease is passed down by a single autosomal dominant gene. FAP has been linked to an APC gene mutation on chromosome 5q during the last two decades (Herrera et al. 1986; Kinzler et al. 1991; Bodmer et al. 1988). One allele of this germline mutation is present in all somatic cells of patients with FAP. This includes colonocytes. If the second APC allele is lost or mutated in an adenocarcinoma-forming colonocyte, individual colonocytes may develop hundreds of adenomatous polyps.

5.2 Sporadic Cancer

These discoveries have expanded our understanding of sporadic colon cancer and syndromic hereditary colon cancer. In the afflicted tissue, a faster colonocyte mitosis and more unpredictable DNA replication are considered to be responsible for the development of colon cancer. Several congenital abnormalities accumulate over time to trigger the transition from normal mucosa to malignant cancer. Fifteen

percent of sporadic colon cancer may be caused by defective mismatch repair genes (Suraweera et al. 2002). Mismatch repair genes are impaired in HNPCC syndrome because of a genetic abnormality. The mismatch repair gene hMLH1 often fails in sporadic serrated adenomas because of DNA hypermethylation. Around 80–85% of sporadic colon cancers are caused by APC mutations (Suraweera et al. 2002). Colon cancer may develop in inflammatory bowel disease via an unexplained mechanism. The spontaneous somatic mutation of APC in colonocytes is thought to cause sporadic adenomatous polyp development. Aberrant crypt foci, the earliest visible dysplastic crypts, often have APC gene mutations early in adenoma development (Hamilton et al. 1994). APC mutations have been found in around half of all spontaneous adenomas (Miyaki et al. 1994). An adenoma is a benign tumor. There are further genetic alterations that must be made before cancer develops.

The normal p53 gene product will halt the cell cycle if the DNA damage is little repairable; if the damage is large and permanent, the cell will undergo apoptosis. It is triggered by radiation or other toxic events to halt cell division and prevent the creation of DNA. When genetic errors are repeated unchecked, a lack of p53 activity may result in the loss of heterozygosity (LOH). The p53 gene is assumed to be crucial in transforming an advanced adenoma into a frank carcinoma. P53 mutations were found in almost half of the colonic lesions with high-grade dysplasia and around 75% of malignancies (Robbins and Itzkowitz 2002).

If LOH is present, it indicates a state of genomic instability due to an overabundance of new mutations (Kern 1989). LOH accelerates the process of carcinogenesis. Several genes have just one copy each in cells with LOH due to chromosomal loss. After a loss of heterozygosity (LOH), a tumor suppressor gene is more likely to lose its normal function. If just one allelic mutation is present, the gene's function is lost.

This technique might reveal a crucial biochemical step in the deactivation of the DCC gene. A receptor in the brain called DCC is essential for encouraging cell death and decreasing tumor development; however, a mutation in the DCC gene has been related to elevated cancer risk. An intermediate adenoma's transition to an advanced adenoma is hypothesized to be influenced by the deletion of the DCC gene (Vogelstein et al. 1988).

5.3 Pathophysiology

5.3.1 Histopathogenesis

Colon cancer develops from polyps in the mucosa of the colon. The histology of a polyp reveals a lot about its development and potential for cancer. Adenomatous and hyperplastic histology are both common. Atypia, nuclear hyperchromatism, or stratification isn't present in hyperplastic polyps despite containing more glandular cells and less mucus (Tsai and Lu 1995). Large, cigar-shaped, palisade-like adenomatous nuclei (Montgomery and Kalloo 2009) are common. Two forms of adenomas are tubular and villous adenomas. Adenomas are created by tubules that branch out and villi that expand out in an orderly way as far as histology is concerned. Adenomas are made up of both components.

According to several epidemiological, clinical, and pathological studies, adenomas are implicated in the vast majority of instances of colon cancer. In the first place, almost one-third of operative specimens with colon cancer are discovered to have synchronous adenomas, a rate much greater than that of age-matched controls who do not have colon cancer (Hiu-Jun Tao and Vadgama 2019). Colon cancer is also significantly increased by adenomatous polyps (Leslie et al. 2002). To complete the picture, adenomatous tissue is often seen with cancerous tissue. FAP patients with hundreds or thousands of adenomatous polyps in their colons will develop colon cancer if they do not receive a colectomy (McConnell 1976). When adenomas are removed, the risk of colon cancer rises to 4% after 5 years and 14% after 10 years (Stryker et al. 1987). Recently discovered molecular evidence supports the adenoma-to-cancer cycle.

Although the data is not definitive, hyperplastic polyps have been associated with colon cancer. A slight increase in the risk of colon cancer may be caused by hyperplastic polyps although this is unlikely to have a significant influence (Cappell and Forde 1989; Blue et al. 1991). In hyperplastic polyps, a large polyp diameter (>1 cm) is a risk factor for malignancy (Jass 2001). Initially characterized as hyperplastic polyps, serrated polyps may be a significant risk factor for colon cancer (Higuchi and Jass 2004). For whatever reason, unlike hyperplastic polyps, which tend to be small and located in the right colon, serrated polyps tend to be significant (Montgomery 2004). These polyps' colonocytes often include high levels of DNA methylation and BRAF mutations (Wynter et al. 2004).

5.3.2 Signs and Symptoms

Colon cancer symptoms are more apparent and frequent when the prognosis is poor but less so in the early stages of the disease. Symptoms such as unwelcome weight loss and a shift in bowel habits are also prevalent (Falterman et al. 1974). However, a sudden change in bowel patterns is much more likely to be caused by colon cancer than a long-term pattern of diarrhea or constipation, even if these are indications of the disease. Less common symptoms include fatigue, nausea, and bloating (Cappell and Goldberg 1992).

There is a direct correlation between the presence of metastases and the size and location of the initial tumor. Compared to cancers of the right colon, those in the left colon are more likely to cause partial or complete blockage of the intestines due to water absorption in the proximal colon (Scarpa et al. 1976). Large exophytic tumors that obstruct the intestinal lumen are also more prevalent. Partially blocked bowels may cause bloating, gas, nausea, and pain. When the stool is partially occluding the obstacle, discomfort may result.

On the other hand, distal cancers may produce significant rectal bleeding because the blood is mixed with excrement and chemically degraded as it travels through the digestive system. There is an iron deficiency anemia in patients with proximal tumors that induce bleeding. Anemia manifests as fatigue, dizziness, shortness of breath, and heart palpitations. There are four clinical symptoms of cancer cachexia:

involuntary weight loss due to anorexia and muscle weakness, ill-feeling about your health, and a feeling of deterioration (Cao et al. 2021).

It is very uncommon for signs of colon cancer to emerge until after the disease has already progressed (Falterman et al. 1974). In certain cases, anemia induced by gastrointestinal bleeding may cause a person to seem pale. In addition to brittle, longitudinally wrinkled, spoon-shaped nails, glossitis, and cheilitis (scaling or fissuring of the lips) are all symptoms of iron deficiency anemia (Anderson 1938). Hypoalbuminemia is characterized by edema, ascites, and anasarca in the limbs. Bowel noises with an unusually high pitch suggest a digestive system obstruction. Abdominal tumors that may be felt are an infrequent symptom of severe sickness. Preliminary tests, such as FOBT, are essential in evaluating suspected colon cancer during colon cancer screening. Digital rectal exams may detect rectal cancer since they are noninvasive. Physical abnormalities such as lymphadenopathy, hepatomegaly due to liver metastases, and temporal or intercostal muscle wasting from cancer cachexia should also be aggressively searched for in patients with peripheral lymphadenopathy. The establishment of Sister Mary Joseph nodes and Blumer's shelves in patients with colon cancer owing to metastases to the periumbilical nodes is an infrequent observation (Cappell 1998).

All patients with a history of colon cancer suspicion should have blood testing for electrolytes and glucose, biochemical liver markers such as bilirubin and AST/ALT, and coagulation profiles. Nearly half of all patients with colon cancer are anemic. In contrast, though colon cancer is relatively common, only a tiny fraction of anemic people has it. Colon cancer screening should be done for older patients with iron deficiency anemia of unclear origin (Ioannou et al. 2002). Hypoalbuminemia is uncommon in colon cancer although it is not unheard of. Malnutrition due to advanced cancer is most often the cause of this symptom.

Regular blood tests reveal that patients with colon cancer typically have normal levels of biochemicals indicative of healthy liver function (Jonsson et al. 1984). If the alkaline phosphatase level is high, patients with impaired liver function are more likely to develop hepatic metastases. Lactate dehydrogenase (LDH) levels may be elevated in patients with colon cancer. Electrolyte imbalances and dehydration are rare effects of diarrhea associated with colon cancer. Nephrotic syndrome, hypovolemia, hypokalemia, and alkalosis may arise from nausea and vomiting caused by colon cancer in rare circumstances.

As previously stated, CEA levels in the blood do not act as a screening tool for colon cancer. It's not very sensitive to light, but it's better than nothing. A small percentage of individuals with early-stage and curable colon cancers have increased levels, but the ranges between these patients and those without colon cancer are relatively close (Sato et al. 1999). In this case, it is a generalization rather than a precise description. Many diseases might produce a surge in carcinoembryonic antigen in the colon or body. An evaluation of cancer prognosis may be made before surgery and an evaluation of postoperative levels. A negative prognosis and an increased chance of postoperative recurrence are linked to serum concentrations above normal (Nicholson et al. 2015). Postoperative serum levels almost often stabilize following complete colon cancer resection, indicating partial resection

(Nicholson et al. 2015). One of the best indicators of cancer return after surgery is a consistent and gradual increase (Nicholson et al. 2015). Patients with this result should undergo a follow-up colonoscopy as soon as feasible to rule out colonic recurrence and metastases.

Acute intestinal obstruction may be caused by exophytic intraluminal growth, which is unusual. Obstruction is prevalent in the sigmoid colon because of its limited lumen and hard stool. When patients come in with stomach discomfort, they report symptoms including constipation, abdominal pain and tenderness, abdominal distention, and hypoactive bowel noises. While malignant tissue may grow into a walled-off abscessed inflammatory mass or tumor with peritoneal symptoms, peritoneal peritonitis is seldom caused by cancerous perforation of the intestinal wall. The integrity of the mucosa may be compromised due to transmural malignant growth or colonic ischemia, increasing intraluminal pressure and increasing the risk of colonic perforation. Colonic obstruction or perforation indicates a poor prognosis in patients who exhibit these symptoms. Due to intestinal dilatation before malignant obstruction or blood vessel invasion, colon cancer seldom causes ischemic colitis (Brandt et al. 1982). Large amounts of rectal bleeding may be caused by malignant ulceration of the colonic mucosa.

5.4 Current Tools

Polyps may typically be safely removed during a colonoscopy. The term "therapeutic endoscopist" refers to a subset of gastroenterologists specially educated to perform non-surgical removal of large polyps. Your doctor may have used a special ink to mark the area of the polypectomy during the colonoscopy (polyp removal). For future colonoscopies, it may be helpful to designate the colon with a marker.

Patients with small polyps should be screened regularly. Patients with more than three polyps or larger polyps should have more frequent follow-up colonoscopies. Find out from your doctor how often you should get screenings. Surgical resection is the most effective way to remove malignant tissue from the colon. Surgeons remove cancer and, if possible, reconstruct the intestine to ensure that your digestive system functions as normally as possible after the procedure. Chemotherapy drugs that travel via blood arteries may destroy cancer cells that have escaped from the tumor and spread elsewhere in the body. Intravenous or oral administration is possible. Chemotherapy may be used at various stages in the treatment of colon cancer. Before surgery, chemotherapy may be used to reduce tumors and avoid more invasive operations. This course of action is referred to as neoadjuvant chemotherapy. More advanced cancers may need chemotherapy in addition to surgery. This kind of chemotherapy is given to patients outside of the hospital in most cases. Chemoradiation is a kind of chemotherapy and radiation treatment that may be provided to patients after surgery. Co-administered chemotherapy and radiation treatment may benefit some people with colon cancer. Radiation therapy is seldom used in the treatment of colon cancer.

5.5 Synbiotics for Colon Cancer

Gibson proposed that prebiotics and probiotics may be combined to generate synbiotics during his discussion on prebiotics (Rastall and Maitin 2002). For one or a few health-promoting bacteria, a synbiotic substance may help the host by boosting their growth and/or activating their metabolism, thereby improving their survival and implantation in the digestive system, according to de Vrese and Schrezenmeir (2008) It is important not to confuse people by describing items with the term "synbiotic," which should only be used to explain the effect of microorganism (Cencic and Chingwaru 2010). When probiotics were extinct, synbiotics were designed to prevent problems if they were not replaced. If probiotic bacteria are fed with synbiotics, they have a better chance of surviving in the upper digestive tract. Assuring effective colonization helps maintain intestinal balance and overall health by encouraging the creation of beneficial bacteria (Peña 2007). The mechanism of action of synbiotics has been defined in Fig. 5.2.

The most often research prebiotics in the battle against colon cancer are fructooligosaccharides and inulin. It has been shown that probiotics are less efficient

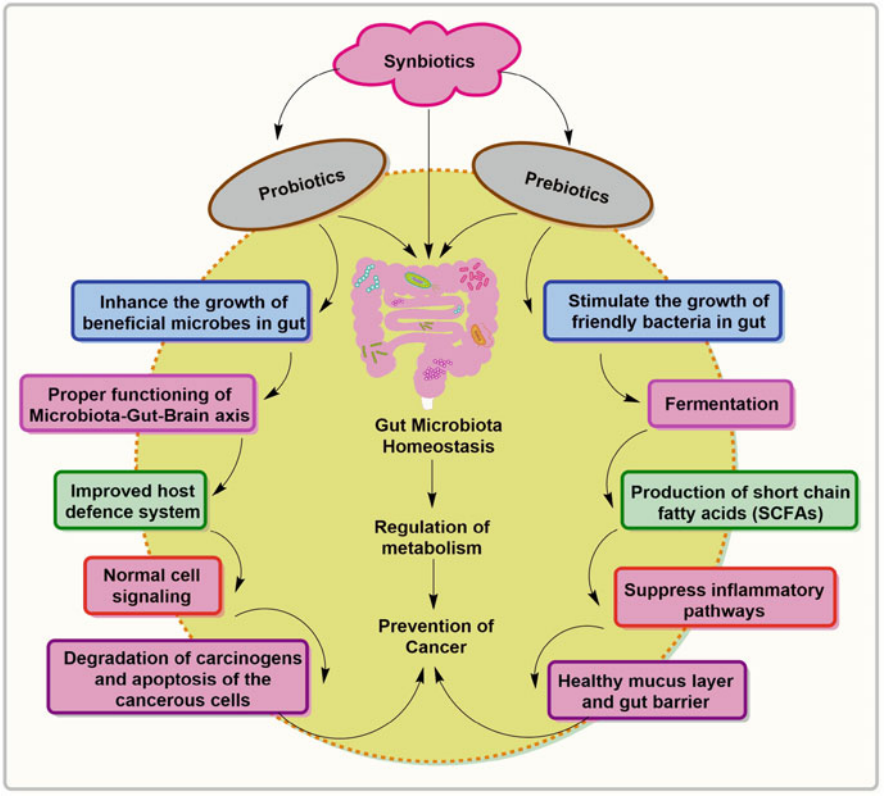

Fig. 5.2 Mechanism of action of synbiotics in colon cancer

in protecting against azoxymethane-induced cancer than prebiotics, according to Femia and colleagues (2002). Although prebiotics reduced colonic proliferation, there was no statistical difference between probiotics and placebos in decreasing malignant colon cancers. The expression of genes encoding enzymes involved in colon carcinogenesis was also studied. *Glutathione S-transferase* and GST pi types in the placenta of rats given the prebiotic alone or in combination with *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb12 had reduced levels. In addition, the inducible nitric oxide synthase (Ahn and Ohshima 2001), which has been associated with colon tumor development and progression, was reduced in the tumors of the rats treated with prebiotics. Researchers also looked at cyclooxygenase-2 and cyclooxygenase-2 inhibitors as possible cancer-preventative enzymes (DuBois et al. 1996). Contrary to expectations, cyclooxygenase-2 expression was higher in the cancerous tumors of untreated than of prebiotics-treated rats in this study. Prebiotics may inhibit carcinogenesis by altering gene expression; however, the precise processes are unknown (Femia et al. 2002).

Prebiotic fermentation in the colon often results in the production of short-chain fatty acids (SCFA). *Lactobacilli* and *Bifidobacteria* are not known to create butyrate, which suggests that some other species of gut flora may be to blame. When using prebiotics, the amount of butyrate that is produced in the colon is influenced (Liong 2008). Because of its importance in fostering phenotypic heterogeneity in colorectal cancer, butyrate accounts for less than 5% of the whole SCFA pool (Reddy 1999). Due to decreased colonic cell proliferation and differentiation induction in colonic epithelial cells, clinical investigations for the treatment of ulcerative colitis with butyrate have risen (Reddy et al. 1997). Although it is known to be an effective growth inhibitor and inducer of apoptosis, it is also thought to have favorable effects in reducing risk factors connected to the development of colon cancer (Kotunia et al. 2004). Treptow-van Lishault and colleagues (1999) identified butyrate, a metabolite that may help detoxify electrophilic and oxidative stress-related chemicals, by fermenting gut microbes on retrograded, high-amylose starch. In the fight against carcinogen-induced colon cancer, butyrate, microflora, and prebiotics can activate enzymes (Wollowski et al. 2001).

In both in vitro and in vivo tests, researchers have shown that a Bifidobacterium strain and fructooligosaccharides, when combined with the correct prebiotics, boost the lifespan and activity of the organism (Gibson and Roberfroid 1995). It is not just probiotic bacteria that synbiotics support, but also new strains of probiotic bacteria that can establish themselves and survive. Colon carcinogenesis was significantly decreased in rats given Bifidobacterium-oligofructose compared to the treatment of either medication alone (Gallaher and Khil 1999). Research conducted by Rafter et al. (2007) on cancer patients and 43 polypectomized individuals assessed the impact of synbiotics in reducing cancer risk factors. Probiotics and prebiotics used in the synbiotic were *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb12. The synbiotic intervention has been proven to diminish the proliferation of colorectal cells and the potential of feces to trigger necrosis in colonic cells, two intermediate signs of colorectal cancer, by scientists. Polypectomized persons, on the other hand, have a better epithelial barrier function. Polypectomized individuals were found to

have lower levels of genotoxicity in their colonic samples after their therapy. Even while scientists aren't sure precisely how the synbiotic intervention affected colonic bacterial ecology and, by extension, metabolism, they hypothesize that it did.

Probiotics and synbiotics have been shown to reduce the incidence of colon cancer in humans. Recurrence of atypical colonic polyps was decreased in 398 participants who took *Lactobacillus casei* for 4 years (Ishikawa et al. 2005). Colon cancer risk was reduced in 37 cancer patients and 43 people with polyps after taking a combination of probiotic *Lactobacillus* GG and *Bifidobacterium lactis* B12 and prebiotic inulin (Rafter et al. 2007; Pool-Zobel 2005). DNA damage, colonocyte cell proliferation, and fecal water genotoxicity (a sign of colon cancer risk) were reduced in poly patients who underwent synbiotic therapy (Lee et al. 2006). The release of interleukin-2 by peripheral blood mononuclear cells was reduced, and the interferon production was increased in cancer patients who ate synbiotics (Rafter et al. 2007). According to these findings, adult use of synbiotics may lower their chance of developing colon cancer.

There was a significant reduction in the expression and proliferation of GST placental enzyme pi, inducible NO synthesizer, and cyclooxygenases-2 enzymes when fructans and *B. lactis* were combined in a synbiotic manner (GG) (Roller et al. 2004a). Synbiotic inulin, oligofructose, *L. rhamnosus*, and *B. lactis* were shown to have anti-tumorigenic effects in mice although PBMC and Peyer's patches had the most effect (PP) (Femia et al. 2002). Even while prebiotic supplementation alone exhibited significant impacts on gut immunity, synbiotic probiotics were most effective. In treating colon cancer, it has been proven that prebiotics and probiotics, which are components of synbiotics, are critical. After AOM treatment, NK-cell-like cytotoxicity reduced significantly in control, probiotic, and prebiotic supplemented groups. There was no evidence of the PP cytotoxicity associated with natural killer (NK) cells in the synbiotic-supplemented group instead of the control group (Roller et al. 2004b). The IL-10 and interferon—generated by synbiotic and prebiotic-fed PPs were higher, and the interferon—produced by these PPs was lower. More than a third of rats exposed to carcinogens got synbiotic treatment, which has been demonstrated to boost the immune system and reduce colon malignancies (Roller et al. 2004b).

Synbiotics seem to outperform probiotics and prebiotics alone in preventing or treating colon cancer. Probiotics and prebiotics mixed with *Lactobacillus* and *Bifidobacterium* strains of GOS, FOS, and inulin were more beneficial than probiotics alone. In vivo studies need more work to determine which strains work well together, the lowest effective dose to achieve the intended health benefits, and appropriate biomarkers.

Research indicates that probiotics may help prevent and cure colorectal cancer (CRC). This review was written by Eslami et al. (2019), who focused on putative immunomodulatory pathways in their research. Farag et al. (2020) studied the different probiotic species, additives, and flavor-enhancing chemicals created during the fermentation process of several improved acidophilus milk products. When given to animals with colitis-associated cancer, the probiotic Bifico mixture reduces the amount of *Desulfovibrio*, *Mucispirillum*, and *Odoribacter* and increases

Lactobacillus levels (Song et al. 2018). *Lactobacillus* probiotics that increased the expression of Wnt/−catenin pathway genes in vitro and a mouse model for CRC generated by *N*-nitroso-*N*-methylurethane reduced cell growth in both cell lines from Caucasians in vitro and in vivo (Ghanavati et al. 2020). If *N*-nitrosodimethylamine is ingested or created in the gut microbiota, *Lactobacillus* strains have been demonstrated to detoxify the toxic chemical (Nowak et al. 2014).

Lactobacillus SB27 acidic EPS was the most effective inhibitory agent on HT-29 cells, and apoptosis and G0/G1 cell cycle arrest were also detected (Di et al. 2018). Additional anti-cancer characteristics and their potential for cancer therapeutic use were also revealed by Wei et al. (2018). People with periodontal disease are more likely to develop colorectal tumors if they have the oral germs *Fusobacterium nucleatum* in their mouths. The presence of *F. nucleatum* DNA in CRC tissue might serve as a diagnostic biomarker in the future (Mima et al. 2016). Immunosuppressive properties of *F. nucleatum*, which impacts T cells and chemokines, may help protect CRC from the assault of the immune system (Shuwen et al. 2019).

Intestinal carcinogenesis, a disease of the lipid-digesting system, is connected to changes in the gut microbiota or bile acid metabolism. According to Liu and colleagues, the bile acid microbiota axis may be targeted using probiotics to prevent and treat colorectal cancer (Liu et al. 2020). As a result, they looked into the involvement of bile acid receptors.

An over-the-counter probiotic decreased the number of aberrant crypt foci (ACF) and the number of malignant neoplastic lesions in rats with chemical-induced CRC and concomitant 5-fluorouracil treatment (by 40% in tubular adenoma, 40% in carcinoma in situ and 20% for low-grade adenocarcinoma) (Genaro et al. 2019).

Lactobacilli that produce vitamin 9 (folate) and vitamin B2 (riboflavin) have been shown to reduce unpleasant reactions in people with chronic inflammation without impacting nutrition, according to a study by LeBlanc and colleagues (2020).

To develop effective probiotic therapy to prevent and manage the occurrence and development of CRC, a screening to select the best probiotic strains must be carried out (Sivamaruthi et al. 2020). According to Settanni et al., in a network of intestinal bacteria and T cells connected to CRC, *Lactobacilli* administered per os in CRC animal models decreased oxidative stress, changed gut microbiota, controlled apoptosis, and modulated immunomodulation (Settanni et al. 2020).

An anti-cancer chemical and antioxidant enzymes bind ROS, release small molecular weight antioxidants (SMWA), and form chelates with transition metals to fight cancer. These chelates interact with proteins that control the cell cycle, limiting cancer cell growth, and weakening cancer cells' resistance to apoptosis by activating procaspases and downregulating the antiapoptotic B-cell lymphoma 2 protein (Bcl2) (Bcl2) (Nowak et al. 2019). TNF-alpha and cyclooxygenase-2 levels in mice fed the bacteria were much lower than those of mice on a normal diet, and the bacteria produced an 80% increase in the mortality of human Dukes' type B colon cancer cells compared with normal intestinal cells. A combination of EGFR, HER-2, and PTGS-2 inhibitors led to a substantial anti-cancer impact in CRC mice (Parisa et al. 2020). Anti-tumor immune responses were increased in C57BL/6 mice after intravenous and oral treatment of *B. bifidum*, a probiotic, respectively. When

intravenously administered to tumor-infected mice, *B. bifidum* has never been demonstrated to exhibit immunomodulatory action (Abdolalipour et al. 2020). Zan Probiotics were protected by microencapsulation throughout the manufacturing process, ensuring their long-term viability in transport, storage, and throughout the whole supply chain (Zandu et al. 2020).

CICC 6074's S-layer protein was able to destroy colon cancer cells. The death receptor pathway and mitochondrial pathway genes were upregulated in the HT-29 cells, resulting in an increase in apoptosis and a decrease in cell invasion. The HT-29 gene is found in cells (Zhang et al. 2020). TNF-, IL-6, and IL-1 levels were reduced in rats treated with 2.5% *L. acidophilus*-fermented germinated brown rice, which reduced CRC preneoplastic lesions. This fermented rice product might be used as a dietary supplement to help prevent colorectal cancer (CRC) (Li et al. 2019b). *L. acidophilus* and/or *Bifidobacterium lactis* in conjunction with germinated brown rice increased antioxidative capability and decreased the synthesis of sialomucin, according to Lin et al. SIM-ACF triggered apoptosis in rats with colon cancer, according to a study (Lin et al. 2019). To test the anti-cancer benefits of prebiotic djulis and *L. acidophilus* per gram in rats given 1,2-dimethylhydrazine and sodium dextran sulfate, researchers gave the rats a combination of the two (Lee et al. 2020). It was found that the combination of ginger extract and L. acidophilus was effective in Wistar rats in reducing lipid peroxidation, increasing catabolism, and restoring colonic permeability and decreasing gut inflammation via the downregulation of COX 2, inducible NO synthase (i-NOS), and regulator oncogene expression (Deol et al. 2018). Mice administered 1.5-g powders of *L. acidophilus* and *B. bifidum* for 5 months saw a decrease in oncogene expression, whereas tumor-suppressor miRNAs and their target genes were elevated (Heydari et al. 2019). In male BALB/c mice, an AOM dosage of 15 mg/kg s.c. raised colon cancer risk by 74%; however, the risk was reduced by 57% and 27% with *L. acidophilus* and *B. bifidum*, respectively.

For example, tumor markers such as CEA and CA199, IFN- and IL-10 blood levels, the number of cells CD41 and CD81 dropped considerably after *L. acidophilus* probiotic administration on mice colon cancer (Agah et al. 2019). After taking *L. acidophilus* and *B. bifidum* probiotics orally, AOM-induced animals with colon cancer had triglyceride, alkaline phosphatase, LDL-cholesterol, and vitamin D receptor gene expression leptin receptor drastically decreased (Ranji et al. 2019). Inflammatory intestinal epithelial cells may be treated with an adjuvant derived from the anti-inflammatory effects of *Lactobacillus acidophilus* and *B. animalis* subsp. *lactis* via the toll-like receptor 2-mediated nuclear factor B and the mitogen-activated protein kinase (MAPK) signaling pathways, according to the findings of this study (Li et al. 2019a).

Cell-free extracts of *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* at doses of 5 and 8 mg/mL substantially reduced the antioxidant activity of HT-29 cells. Overexpression of caspase-9 and caspase-3 also led to apoptosis (Baghbani-Arani et al. 2020). Compared to a control group of C57BL/6 mice with colon cancer, *Lactobacillus acidophilus, Bifidobacterium bifidum*, and *Bifidobacterium bifidum*

reduced tumor size and tumor number while increasing the abundance of *Lactobacillus* and *Bifidobacterium* (Mendes et al. 2018).

FOLFOX (an amalgam of the medications folinic acid (leucovorin) "FOL," 5-fluorouracil ("F"), and oxaliplatin ("OX")) was shown to not affect the anticancer activity of *L. rhamnosus*, a probiotic, in mice with CRCs (Chang et al. 2018). Taking *L. rhamnosus* supplements for a long time changed the gut microbiome significantly. There was a significant rise in the relative abundance of *Limnobacter* and Turicibacter Sales, *Enterococcus* and *Vagococcus*, Helicobacteraceae and Rikenellaceae, *Roseburia* and Doren, Anaerostipes and *Coprococcus*, *Oscillospira* and *Rumi*. Amino acid transport and metabolism were enhanced by *L. rhamnosus* supplementation (Gamallat et al. 2019a). According to Huang et al. (2019) *L. rhamnosus* protects rats against colon cancer by reducing inflammatory and angiogenesis gene expressions and increasing apoptotic gene expression. *L. rhamnosus*, for example, inhibited human colon cancer cell lines Caco-2 and HT-29 (Sharma et al. 2020). In Sprague Dawley rats, oral *L. rhamnosus* gavage reduced ACF numbers and gut microbiota structure, composition, and functions considerably (Gamallat et al. 2019b). Cell growth was suppressed in vitro by 600–800 gEPS/mL of *L. fermentum* YL-11 galactose, researchers observed (Wei et al. 2019).

Glucuronidase activity in rat feces was decreased by 57% and 50% when *L. fermentum* or *P. acidici* TISTR, manno-oligosaccharides, and rice syrup-oligosaccharides were added to the three lactobacilli strains; the most significant impact was shown when *L. plantarum* DSM 2648 was administered with *P. acidici* EPS (Chaiongkarn et al. 2019). Colon cancer cells from three different types were repressed by CLNA isomers made from *L. plantarum* ZS2058, and the antiproliferation impact of CLNAs in Caco-2 cells was connected to oxidative stress. Even though crucial apoptosis-related proteins were unaffected, evidence suggests that CLNA1 and CLNA2 pyroptosis pathways activate caspase-1 and induce Caco-2 cell death (Ren et al. 2020). As data published, the anti-colon cancer activity of *L. plantarum* C70 (KX881779), EPS from camel milk, was 88.1% when given 10 mg/L (Ayyash et al. 2020). *L. plantarum* was produced in HT-29 cells from fermented durian (Tempoyak), a traditional Malaysian condiment that has a high adhesion index (15910), a high pH tolerance (2.0), and a bile salt tolerance of 0.3%. Bacterial cells, both alive and the growth retard by HT-29 cells (Ahmad et al. 2018).

While *L. plantarum* MBTU-HK1 lowered cholesterol levels in male Balb/c mice, acacia gum boosted protein, mineral, and immunoglobulin levels (a prebiotic). When these two medications were combined, TNF-α and pro-carcinogenic fecal enzyme activity from bacteria decreased, suggesting that they might help prevent the beginning of CRC (Honey Chandran et al. 2019).

5.6 Conclusion

Since cancer has become a part of our daily lives, virtually no family in the Western world does not have a cancer patient in it. A sedentary lifestyle, a preference for fast food, a lack of exercise even in youth, and an elevated stress level are all likely factors. A small number of cancers are curable, while most cancers cause mortality in their initial stages or after a recurrence in the patient. Anti-cancer treatments now on the market tend to create major side effects that might be reduced by the use of many other medications. Taking probiotics, which are made up of the so-called good bacteria, may help prevent cancer, and a well-balanced diet should surely include enough prebiotics to support the positive effects of probiotics on human health. Probiotics have been proven to have a favorable impact on a wide range of physiological processes, including increasing the effectiveness of treatment or lessening the adverse effects of anti-cancer medications and radiation therapy. When it comes to dietary supplements, it is becoming more common to see probiotics incorporated with other bioactive substances, such as vitamins, to enhance their ability to improve the health of the body. This means that probiotics in nanoformulations may be more effective in treating the side effects of existing cancer treatments and may serve as an adjunct to therapy, but it should be emphasized that probiotics do not cure any type of cancer on their own, even if they are given to healthy individuals.

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Synbiotics in Cervical Cancer

6

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6.1 Introduction

Cancer is the most significant global health issue and cause of death that people can experience in life. The prevention and treatment strategy has become more advanced in the last few decades although the suffering from this deadly condition rapidly growing due to various factors and therapeutic challenges. Therefore, researchers

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from every corner of the world are trying to progress in advanced therapeutic approaches. Even with the fact that cancer development depends on various parameters like genetic factors and environmental conditions, the immunological system of the organism has a significant role and is nearly linked with beneficial bacteria including commensal flora bacteria that exists in the digestive, tract. Cervical cancer (CC) is the second greatest cause of mortality along with women worldwide, with around 80% of instances occurring in developing countries. CC is most diagnosed in the fifth decade of life, earlier than the standard age at diagnosis of breast, lung, and ovarian cancers (Waggoner 2003). Although, women (>55 years old) are more susceptible to CC, commonly because of a more progressed disease at diagnosis (Sung et al. 2000). The main causative agent of CC progression is human papillomavirus (HPV) which is the source of sexual activity. More than 90% of squamous cervical malignancies include HPV DNA, according to research (Bosch et al. 1995). Most incidents occur in developing nations because of no adequate screening measures in place. HPV infection, smoking, and immune system dysfunction are all risk factors. During the early stage of tumors, women can be cured, however, morbidity throughout a lengthy period of time the therapeutic approach is quite general. There are numerous kinds of bacterial strains in the human body, which gives beneficial effects on health (Nami et al. 2014a, b); therefore, there must be a link between the host's immune system and the bacteria found in the urogenital tract (Riaz Rajoka et al. 2017). Alteration of healthy microbiota causes cancer of cervical by permitting contagious components to progress in this region. Currently utilized chemotherapeutic bioactives lead to cytotoxic events in cervical cancer patients and adverse effects due to therapies that are unavoidable (Kuku et al. 2013). Thus, it is required for anti-tumor actives that are least toxic and show less toxicity than other active drugs. Additionally, some therapeutics are used to inhibit the after-effects of cancer of cervical management which are efficient in increasing patients with cervical cancer have a worse quality of life (Yu et al. 2017).

6.2 Pathophysiology

The predominant etiologic agent has been outlined as high human papillomavirus (HPV). Extensive cervical dysplasia or carcinoma-in-situ is the commonest precursor to invasive cervical cancer. Cervical cancer is a sexually transmitted illness provoked by exposure with human papillomavirus (HPV) strains that aggravate cancer. HPV annihilates the healthy DNA genetic composition including consistent cell multiplication procedures of the tissues in the cervix, promoting uncontrolled cell division involving finally a huge malignant innovation. Invasive cervical malignancies worldwide incorporate oncogenic HPV DNA in over 95% of cases.

Persistent infection with high-risk oncogenic HPV strains is the pivotal etiologic consequence for the genesis of cervical cancer (Zur 2009). Immune dysfunction, mutagen exposure, including hormonal fluctuations is all established risk factors for cervical cancer. According to identical investigations, genetic origin exerts a little impact in the progression of cervical cancer. Prior sexual activity, numerous sexual partners, exposure to other sexually transmitted illnesses, cigarette smoking, oral

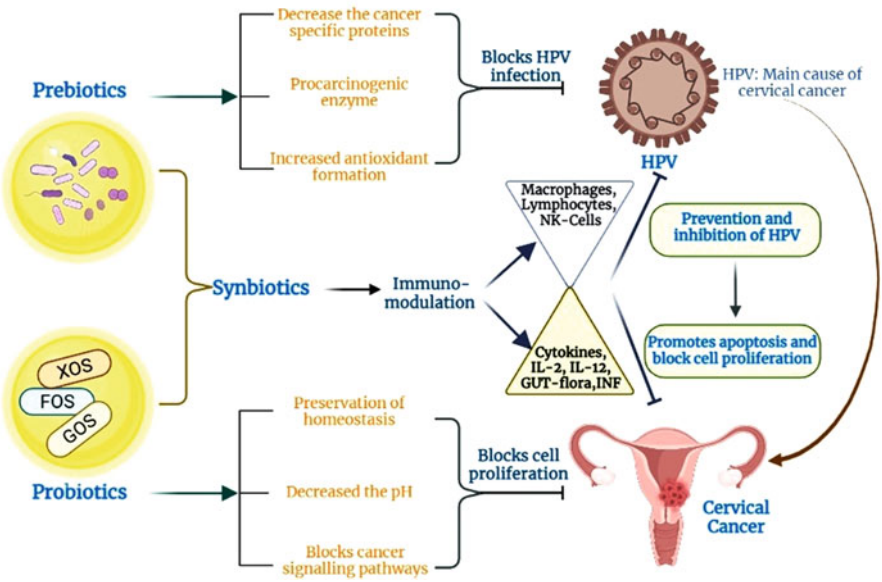

Fig. 6.1 Implications of synbiotics in the management of cervical cancer

contraceptive utilization, infection with the human immunodeficiency virus, including immunosuppressive medication therapy are the greatest prominent concerns (Chauhan et al. 2009). Epidemiological investigations have demonstrated that there is a substantial significance among the occurrence of cervical cancer including metabolic variables abnormalities. In essence, metabolic factors serve a vocation in the emergence of cervical cancer, but the exact mechanism by which these distinct or integrated metabolic factors interact with cervical cancer emergence persists elusive (Ashique et al. 2022). The preponderance of HPV infection is ephemeral. When chronic HPV infection persists, it is anticipated that it requires an average of 15 years from the time of initial infection to the coalescence of cervical intraepithelial neoplasia (CIN) or, subsequently, invasive cervical cancer (Lea and Lin 2012). Figure 6.1. discusses the implications of synbiotics in the management of cervical cancer.

6.3 Histopathogenesis

The most prominent histology of cervical cancer is squamous cell carcinoma, which estimates for around 80% of cases, with adenocarcinomas contributing for about 20% of cases. Small cell carcinomas, melanoma, including lymphoma are less pervasive histologies. Squamous cell carcinoma including adenocarcinoma is the two most frequent histologic features of cervical cancer. The squamocolumnar junction are where squamous cell cancer of the cervix invariably begun. More than 70% of all cervical cancers are squamous cell carcinomas.

Squamous cell carcinoma has declined in incidence during the preceding 30 years, but adenocarcinoma has elevated. This is most likely connected to Pap smear screening for premalignant including fatal illnesses of the cervix (Simcock and Shafi 2007).

6.4 Signs and Symptoms

Postcoital or unusual vaginal bleeding, watery vaginal discharge, involving physical evidence of venous, lymphatic, neural, or ureteral compression are all popular symptoms intimately familiar with cervical cancer. Cervical cancer is typically identified after a physical exam including histologic investigation of cervical specimens.

6.5 Recapitulation of Probiotics, Prebiotics, Including Synbiotics

All synbiotics have been extensively researched recently for their purported health benefits. Dietary supplements have been shown to affect, modify, or restore the native gut flora. Additionally, they support keeping the digestive environment in top working order. The most widely used probiotic strains are *Lactobacilli*, *S. boulardii*, *Bifidobacterium*, and *Bifidobacterium coagulans*. When combined with probiotics, synbiotics' most often utilized fibers may increase probiotic persistence, including prebiotics like "GOS, XOS, FOS, or fructans," and inulin (Pandey et al. 2015). Gibson initially proposed the idea of prebiotics and speculated about the extra advantages that would attain if prebiotics and probiotics were combined to create synbiotics (Vrese and Schrezenmeir 2008). A synbiotic product helps the host to increase the survivability of the implantation of live microbial dietary supplements in the gastrointestinal tract by specifically increasing the development of stimulating the metabolism of one or a small percentage of health-boosting bacteria. The word "synbiotics" should only be used to describe products in which the prebiotic compound(s) benefits the probiotic bacteria solely since it indicates a synergistic interaction (Cencic and Chingwaru 2010). They aid in synthesizing nutrients or increase their bioavailability; certain probiotics have been shown to have antioxidant effects when appearing as whole cells. Probiotics have also reportedly been shown to help with lung infections, AIDS, cancer, and allergy problems (Harish and Varghese 2006).

6.6 Recent Therapy for the Cancer of Cervical Treatment

Management of cancer of cervical approach turns on various components including phases of illness, histopathology of the tumor, local including distant metastases, tumor degree proliferation (grade-G), and initial lesion size. Currently, three curative

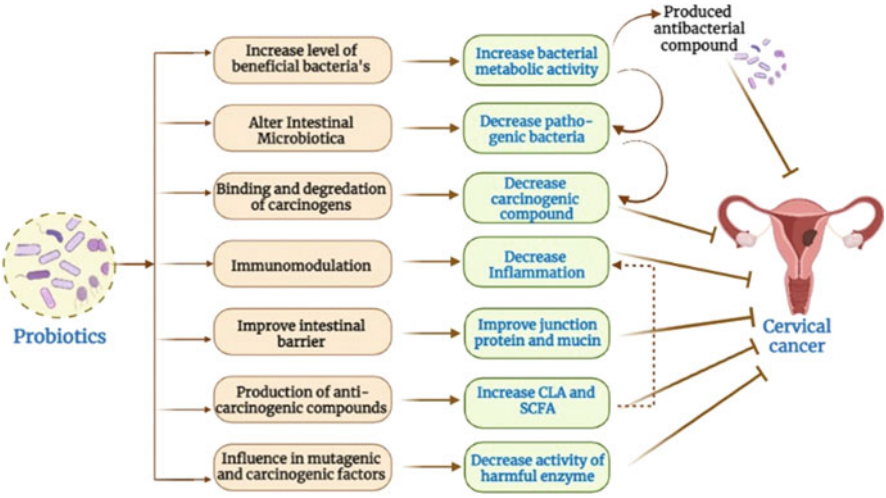

Fig. 6.2 Potential mechanisms of probiotics action in the prevention of cancer development

approaches are being mainly considered surgery, hormone chemotherapy, including radiotherapy (Janicek and Averette 2001). The existing therapeutic approach is predicated on the use of a multidisciplinary team. Numerous restorative interventions were employed in the initial stages, including surgery, radiotherapy, neoadjuvant chemotherapy, with fertility sustainability surgery. Concurrent chemoradiation therapy (CCRT), which utilizes cisplatin alone or in conjunction with other bioactives, is the most frequent or favored modality. There is a paucity of complete reply in about 30–40% of cases (Kumar et al. 2018). Individuals with bone metastasis, lung metastasis, multiple brain metastases, or solitary brain metastasis can be cured with a diverse array of therapies, including craniotomy, chemotherapy with surgery, stereotactic radio-surgery coupled with radiotherapy, "chemotherapy including palliative brain radiation," among others (Li et al. 2016). As per findings, women with a positive HPV test have a 65% higher risk of cancer of cervical than women with a negative outcome. Women with chronic HPV infection also have an elevating risk of cancer of cervical. Among 2 years, the normal Papa findings is more prone to progress into "cervical intraepithelial neoplasia II or III (CIN II and CIN III)" in women who have a negative Human Papillomavirus test (Al-Daraji and Smith 2009). Figure 6.2 represents the potential mechanism of probiotics.

6.7 Limitations Associated with Current Therapy

The advanced therapeutic strategic approach for prevention, screening, diagnostic, and treatment of cancer somewhere developed challenges for the patients. Patient and system-dependent limitations must be considered as part of any cervical cancer

control program. Limited human volunteers, few clinical trials, and infrastructure are the most significant barriers to cervical cancer treatment methods. Early-stage, locally progressed, or metastatic cervical cancer can be detected using a multidisciplinary approach that includes gynecological oncology, medical oncology, imaging, pathology, radiation oncology, and palliative care. Economical investment in cervical cancer research initiatives in low- and middle-income countries should include effective recruiting programs that engage community women in cancer screening and detection procedures. Although cervical cancer is preventable and curable, there are significant hurdles to cervical control, which may need extensive coordinated and sustained action by numerous stakeholders before progress can be made (Randall and Ghebre 2016).

6.8 Role of Synbiotics on Cancer Cells

Several research have been conducted to evaluate the health benefits linked to pro- and prebiotic additives that sometimes contain live microorganisms. The main focus of research is based on the efficacy of synbiotics for the management of various types of cancer. Currently, many researchers have focused on the assessment of synbiotics to treat cervical cancer, a major cause of death in gynecology globally, mainly in growing countries. Currently, several clinical studies already described the efficiency of probiotics against cervical cancer although some certain challenges like quantities and dose of drug, species of bacterial, and therapy duration are somewhat inaccurate. Pre- and probiotics in combination with other therapeutics provide several beneficial effects like anti-obesity antidiabetic, anti-pathogenic including anti-inflammation (Bahmani et al. 2016; George Kerry et al. 2018) and also help in boosting the antibody responses, reducing mononuclear cell progression, etc. (Kankaanpää et al. 2003; Bodera and Chcialowski 2009). Several studies were conducted on the application of synbiotics for the detection, prevention, or management of cancer purpose. Along with their direct association with cancers they can also be utilized as active agents; for altering other therapeutic including diagnostic approaches (Kailasapathy and Chin 2000) have described some following mechanisms of synbiotics like the initiation of the immune system, altering the inhibiting pro-carcinogens including carcinogens, reducing bacteria that govern the conversion of pro-carcinogens to carcinogens, including inhibiting intestinal pH. Various studies have reported that probiotics have a significant role in various biological mechanisms that are linked to cancer initiation (like apoptosis, inflammation, oxidative stress, metastasis including proliferation) (Saber et al. 2017). It was found that probiotic supplements can be used to inhibit liver cancer because of they are able to decrease the aflatoxin exposure dose which is biologically efficient (El-Nezami et al. 2006). In preclinical studies, important anti-tumor agents like probiotics and their metabolites (like pyridoxine; butyrate, with SCFAs) give energy to the colon's cells while regulating the intestine's acidic environment, lowering the production of high levels of secondary bile acids, and enhancing cancer cells' ability to undergo apoptosis (Kahouli et al. 2013). Among all the metabolites butyric acid

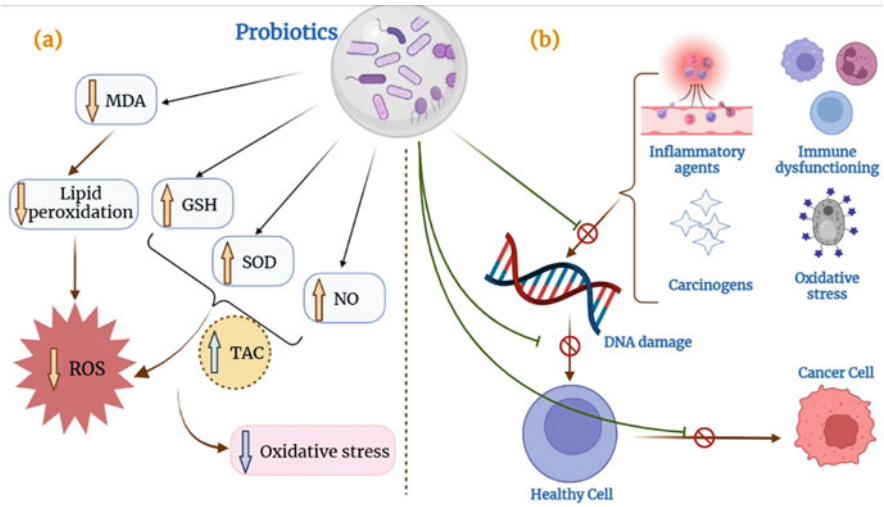

Fig. 6.3 (**a**) Mechanism of ROS inhibition by synbiotics in cervical cancer; (**b**) probiotic bacteria prevent DNA damage and mutation, prevent the formation of cancer cells

maintains the progression, apoptosis, and division and large amount of butyrate is generated by metabolism of intestinal cell. It was found that in comparison with healthy individuals, there is an apparent inhibition of this kind of acidic content in the feces of individuals suffering from colorectal tumor (Macfarlane and Macfarlane 2003). Though SCFAs are produced from intestinal flora, because of several dissimilarities, the amount generated cannot be adequate to block the progression of colorectal cancer. As an outcome, taking probiotics may enhance the production of SCFAs, which can hinder pathogen growth. Several in vitro including in vivo studies revealed that synbiotics are a prominent approach to managing cancer (Raman et al. 2013). Prebiotics are the fermentable, non-digestible food components that boost host energy and minimize cancer through the "mechanisms of fecal bulking, colonic pH alteration, carcinogen binding to bacteria, modification of xenobiotic-metabolizing enzymes, altered gene expression in the feces and caecum, and modulation of immune responses" (Harris and Ferguson 1993). Certain host-beneficial probiotics and prebiotics are chosen to increase the survival, development, and functionality of the chosen probiotic strain. The host's gastrointestinal system's resident beneficial bacteria is thereby increased due to the prebiotics. A single or multi-strain probiotic and a proper combination of prebiotics, where the latter favors the former and produces a synergistic response, must be included in an ideal synergistic synbiotic supplement. It must encourage the growth of naturally occurring good bacteria and limit the growth of cancer-causing germs (Kolida and Gibson 2011; Kondepudi et al. 2012). Figure 6.3 represents the Mechanism of ROS inhibition by synbiotics in cervical cancer and probiotic bacteria prevent DNA damage and mutation, prevent the formation of cancer cells.

6.9 Impact of Probiotics on Cancer Cells

Wang et al. (2018) reported that *Lactobacillus supernatants* (LS), *L. gasseri*, *L. crispatus*, including *L. jensenii* can suppress the progression of cells of Caski which resulted in several changes in morphological features. By using the number of S phase cells involving incubation on cells with LS was reported to be increased significantly, whereas the amount of G2/M phase cells was reduced. There are numerous genes involved with HPV infection and among them; the gene E6 and E7 are mostly coded by HPV. These two genes are required for cancer development and are closely related to the tumor suppressors p53 and pRB (Yim and Park 2005). Treatment with LS lowers the expression of "CDK2, cyclin A, and HPV oncogenes (E6 and E7)." *Lactobacillus Plantarum* bacteria were found in the vaginal discharge of adults, including teenage girls. These bacteria were shown to have effective probiotic qualities, such as an anticancer effect against the HeLa carcinoma of the cervix lining (Nami et al. 2014a, b). Another study on HeLa cell lines found that isolated "*Lactobacillus* strains (i.e., *Lactobacillus casei* SR1, *Lactobacillus paracasei* SR4, and *Lactobacillus casei* SR2) secreted from breastmilk had notable probiotic qualities like antioxidant capacity, antibiotic susceptibility, low pH resistance, and resistance to high levels of bile salts" (Sungur et al. 2017). G10 and H15, human vaginally isolated *L. gasseri* strains, were efficient against the advancement of HeLa cells, and *L. gasseri* was also able to block TNF- and boost IL-10, resulting in an anti-inflammatory action against cervical cancer. *Lactobacillus* exopolysaccharides have been discovered to have cytotoxic impact on cells of cancer and to suppress tumor cell development (Liu et al. 2011a, b). Another study demonstrates that the treatment of HeLa cells by *Lactobacillus crispatus* and *Lactobacillus rhamnosus* decreases the transcription of the "CASP3 gene" and MMP-2 with MMP-9, resulting in a metastatic antagonistic effect (Nouri et al. 2016). As per the findings, "*Bifidobacterium adolescentis* SPM1005-A" exhibits antiviral action in the SiHa cell line of cervical, which displays HPV type 16, or can deter cancer from proliferating (Table 6.1) (Cha et al. 2012).

6.10 Mechanisms of Action: Probiotics and Cancer

Although there is no specific established mechanism behind the role of synbiotics linked with anticancer efficacy. But it was found a link between a healthy gut microbiota environment with several types of metabolic diseases, and thus synbiotics are considered to have a significant role in maintaining a healthy micro-environment in this process. Generally, probiotic bacteria offer a promising role in the preservation of homeostasis and conserving a controllable physicochemical environment in the colon. Decreased pH due to the over-production of bile acids in feces is responsible for affecting colonic epithelium thus resulting in colon carcinogenesis (Jia et al. 2017; Bernstein et al. 2005). Not only in the alteration of pH including bile acid profile probiotics along with prebiotics (*B. Bifidum* and *L. acidophilus*) were found to be a prominent approach for the management of

Table 6.1 Probiotics have been investigated for their potential to reduce the gastrointestinal adverse impacts of cancer of cervical therapy (Jahanshahi et al. 2020)

Varied probiotics	Dosage	Probiotic consumption span	Results	Reference
Bifidobacterium animalis subsp. *lactis* BB-12 plus *L. acidophilus* LA-5	1.75 billion lyophilized live bacteria (t.i.d.) in single capsule	From the initiation to the end of radiotherapy	Chronic frequency of radiotherapy-related diarrhea decreased	Linn et al. (2019)
Bifidobacterium bifidum plus *L. acidophilus*	2×10^9 *Bifidobacterium bifidum* b.i.d. + *L. acidophilus* units	Initiation 7 days before starting radiotherapy involving continuing per day at radiotherapy time	Consistency of stool was found to be better and the frequency of anti-diarrheal decreased	Chitapanarux et al. (2010)
L. casei DN-114001	96 mL yoghurt liquid with 10^8 CFU/g *L. casei* DN114001 t.i.d.	The first 7 days before introducing radiotherapy and continuing every day during radiotherapy	Better stool consistency but no reduction in the induced diarrhea via radiotherapy	Giralt et al. (2008)
VSL#3	One sachet with 450 billion bacteria per gram, comprising three *Bifidobacteria* strains, four *Lactobacilli* strains, including one *Streptococcus salivarius* t.i.d.	From the initial day of radiation therapy until the radiotherapy scheduled cycles end	Radiotherapy-associated diarrhea decreased with per day bowel movements	Delia et al. (2007)
Symbotic	1×10^7 (CFU)/g *Lactobacillus* biogel *acidophilus* NCFM, 1×10^6 CFU/g biogel of *Bifidobacterium lactis* Bi-07, with blue agave inulin t.i.d.	In 7 weeks	Fecal calprotectin including frequency with reduced frequency of vomiting	De Loera Rodriguez et al. (2018)
L. casei LC9018	0.1 mg twice a week or 0.2 mg once a week during radiation therapy, then 0.1 mg every 2 weeks or 0.2 mg every month for the next 2 years, or until the tumor recurs	6 months after radiation therapy	Decreased immunity against tumor induction	Okawa et al. (1993a, b)

cancer (Biasco et al. 1991; Lidbeck et al. 1991). Several probiotics also help to maintain the balance between the healthy environments of natural intestinal microflora including their metabolic function. Various putrefactive bacteria, including *Clostridium perfringens* or *Escherichia coli* are often abundant in the gut which have been implicated to the generation of carcinogenic components when enzymes are applied (nitroreductase, azoreductase, including b-glucuronidase). According to preliminary investigation undertaken by Goldin including Gorbach in the late 1970s, ingesting milk fermented commodities (*L. acidophilus*) has a beneficial impact in inhibiting putrefactive bacteria by reducing the concentration of the deleterious enzyme (Goldin and Gorbach 1977). Various relevant research studies reported the pragmatic impact of various strains of probiotic on the function of enzymes bacteria involved in tumorigenesis in humans (Kim and Jin 2001). Gut microbiota generate or metabolize a multitude of important compounds that contribute in sustaining homeostasis including averting cancer. As a consequence of the fermentation of fiber-rich prebiotics, a specified type of gut microbiota yields short-chain fatty acids (SCFAs) such butyrate, propionate, involving acetate which have substantial influences on the proliferation, immune system, or cell death (Garret 2015). Numerous studies have revealed that the human microbiome is a prominent player in carcinogenesis and an important component of a tripartite "interactome" with the patient and the gut ecosystem (Alexander et al. 2018). Because of its crucial role, investigators are concentrating on how the microbiome may be altered by employing pre-, pro-, and synbiotics for cancer treatment. Certain strategies, such as altered "intestinal barrier function, immunomodulatory, metabolic, and anti-proliferative effects," depend on synbiotics' carcinogenic action.

6.11 Intestinal Membrane Functionality

One of the key components of the microbiota's symbiosis is the physiological separation of the bacteria from the recipient. Carcinoma is caused by structural obstacles between the patient and the microbiome interfering with each other (Schwabe and Jobin 2013). The intestinal barrier is made up of enterocytes and cell junction proteins, immune cells, secretory IgA, and antimicrobial peptides. Several studies reported that the colonic epithelial integrity improves while introducing synbiotics (Liu et al. 2011a, b).

6.12 Immunomodulation

A balanced intestinal microbiome microenvironment is necessary for immune response development and promoting an anti-tumor potential, in both intestine and at distant locations. "0 T-lymphocytes, natural killer (NK) cells, and dendritic cells" among others, may recognize and eliminate damaged or potentially cancerous patient/recipient tissue (Fernandez et al. 1999). The reaction between probiotics with dendritic cells happens through "toll-like receptors" that prompt the T-cell

and NK-cell activities. Various synbiotic ingestion along or with other therapeutics (*Lactobacillus casei* or *Bifidobacterium lactis*) have resulted in improved NK cell response in both rodent models (Ogawa et al. 2006) and human studies (Gill et al. 2001). Various in vivo studies through rodent models resulted in probiotic encouraged natural killer cell potency which may lead to diminish cancer cell proliferation (Lim et al. 2002).

6.13 Metabolism

The metabolic function of microbiota provides an oncosuppressive response through several mechanisms. Bacterial metabolites such as (SCFAs; butyrate, acetate, and propionate) provide significant pro-apoptotic functions, anti-proliferative and anti-inflammatory. The "SCFA butyrate" is the most researched and promotes the activation of Treg cells and decreased expression of NF-κB. According to reports, prebiotic treatment promoted SCFA production, increased *Bifidobacteria* involvement, and provided prevention towards colorectal cancer (CRC) connected to chemically driven intestinal inflammation (Hu et al. 2016). Although, the effects of synbiotic ingestion in humans showed numerous results with regard to enhancing SCFA generation (Phillips et al. 1995). Whereas another, the delivery of synbiotics to healthy adult participants won't result in any alterations in the levels of fecal SCFA (Worthley et al. 2009).

6.14 Impact of Probiotics on Proliferation

Probiotic strains are well-known examples of *Lactobacilli* that have boosted the mortality of tumor cell lines due to their anti-proliferative and pro-apoptotic properties (Iyer et al. 2008) and similar response to murine models (Le Leu et al. 2005). Numerous methods for these mechanism have been suggested. TNF-α and caspase-based apoptosis are produced by altering cell signaling cascades (such as MAPK and NF-κB), whereas SCFAs like butyrate promote an antiangiogenic effect by inhibiting histone deacetylase (Berni Canani et al. 2012). DNA damage is a key factor for cancer cell progression whereas treatment with synbiotics displays antigenotoxic properties in animals exposed to mutagens (Pool-Zobel et al. 1993).

6.15 Limitations of Synbiotics

The significant outcomes given by synbiotic therapy also have some certain challenges due to various factors like few clinical trial studies, a small number of cohort studies, etc. (Kumar et al. 2015). Research findings do not support several bacteria often used as "probiotics" in fermented product items in different nations (Sanders and Klaenhammer 2001). Probiotic bacteria can have negative consequences in addition to beneficial ones, including severe illnesses, harmful

metabolic changes, and excessive immunological activation in immunocompromised individuals (Marteau 2001). As a result, the long-term responsiveness to probiotic delivery methods has to be regulated. All probiotic strains must undergo in vitro and in vivo quality assessments such as "acute, sub-acute, and chronic toxicity studies" (Papadimitriou and Kok 2011). Rash, hiccups, nausea, constipation, and flatulence are very common side effects of probiotics (Islam 2016). There are other probiotic adverse events like systemic infections, dysregulation of metabolic responses, and transferring deleterious genes like resistance to antimicrobial components (Hojsak et al. 2018). *Lactobacillus* has been implicated to endocarditis, sepsis, including liver abscesses in a few rare instances. *Bacillus subtilis* can promote cholangitis, bacteremia, with sepsis, according to investigations (Boyle et al. 2006).

6.16 Oxidative Stress in Cervical Cancer and its Response to Synbiotics

The main responsible factor in cervical cancer is human papillomavirus (HPV), which advances via sexual intercourse; males are considered to be the major mediators in most cases, infecting and developing the disease in women and many adults are unconscious of HPV infection linked adverse effects (Braaten and Laufer 2008). HPV shows CaCx by injuring the DNA although current data concluded that oxidative stress (OS) is a significant factor for initiating cancer growth (Smita et al. 2007). Chemoradiation is a commonly used approach for increasing the survival of patients with CaCx. Free radicals are developed when the levels of antioxidants, get decreased which causes DNA damage, resulting in cell dysfunction and disease progression. ROS is mainly responsible for DNA damage, mutations of tumor suppressor genes, and the promotion of multi-step carcinogenesis (Georgescu et al. 2018). The reactive oxygen species (ROS) is generated by various mechanisms which promote lipid peroxidation and cause the over-production of MDA biomarkers, which alter the cellular function and initiate cancer progression (Ayala et al. 2014). The increase in MDA concentration is responsible for the over-production of ROS because of enhanced oxidative injury in patients having uterine cancer. During the disease progression, the reactive oxygen species (ROS) generation also enhances which initiates lipid peroxidation, and thus cellular membrane degeneration, and DNA damage occurs (Barrera 2012). The disequilibrium between ROS and the antioxidant defense system, which is connected to several non-illnesses, including "cardiovascular, cancer, and diabetes," is primarily caused by oxidative stress (OS) (Jones 2006). Additionally, increased ROS production, damages cell membrane proteins, fatty acids, and nucleotides, which causes cellular disorders (including impaired energy homeostasis, altered signal transduction, DNA synthesis control, DNA and RNA genetic changes, cellular-transportation defects, and decreased immune function (Squier 2001). Various previous research studies have reported that the introduction of dietary supplements showed antioxidant efficacy which inhibits oxidative stress and thus cancer cell progression (Lobo et al. 2010).

6.17 Combination of Probiotics with Other Cervical Cancer Therapies

Cisplatin has been found to be the accepted preferred anticancer drug in patients having progressed cervical cancer (Tsuda et al. 2016). In lung cancer mice models, it was demonstrated that co-administration of cisplatin with *Lactobacillus* bacteria enhanced cisplatin pro-apoptotic including antigrowth responses. *Lactobacillus* co-therapy enhanced IFN-, PRF1 including GZMB expression, culminating in an enhanced cisplatin efficacy. Lactobacillus-based combination medication elevated GZMB, PRF1, including IFN-γ-, expression, culminating a superior anti-carcinogenic potential with platamin (Gui et al. 2015). In animals with an improper intestinal microbiome, PD-L1 inhibition by orally administered "*Bifidobacterium*" probiotics resulted in increased anti-tumor efficacy although this combination therapy also limited tumor development (Sivan et al. 2015). An additional study of 228 individuals having stage III-B cervical carcinoma found that probiotics reduced reactive oxygen species (ROS) and cisplatin-containing biomass-encapsulated vaginal suppositories were more effective at treating cervical carcinoma (Okawa et al. 1993a, b; Negi et al. 2020). Probiotics combined with nanocarriers and anticancer drugs promotes treatment and medication therapy (Table 6.2) (Fig. 6.4).

Table 6.2 Several on probiotics having beneficial effects on cancer cells of cervical cancer (Jahanshahi et al. 2020)

Probiotics	Cell lines	Major outcomes	Ref
Supernatants of *L. jensenii*, *L. crispatus*, including *L. gasseri*	Caski	Reduction in initiation of HPV oncogenes including cell cycle-involved genes	Wang et al. (2018)
Vagina-isolated *L. plantarum*	HeLa	Reduction of progression with initiation of apoptosis	Nami et al. (2014a, b)
Milk-isolated *L. casei* including *L. paracasei*	HeLa	Initiation of apoptosis	Riaz Rajoka et al. (2018)
Vagina-isolated *L. gasseri*	HeLa	Inflammation including cancer cell division decreased with enhanced apoptosis exhibited.	Sungur et al. (2017)
Supernatants of *L. rhamnosus* with *L. crispatus*	HeLa	Progression with metastasis inhibited	Liu et al. (2011a, b)
Bifidobacterium adolescentis SPM1005-A	SiHa	Reduction of E6 including E7 oncogenes	Nouri et al. (2016)

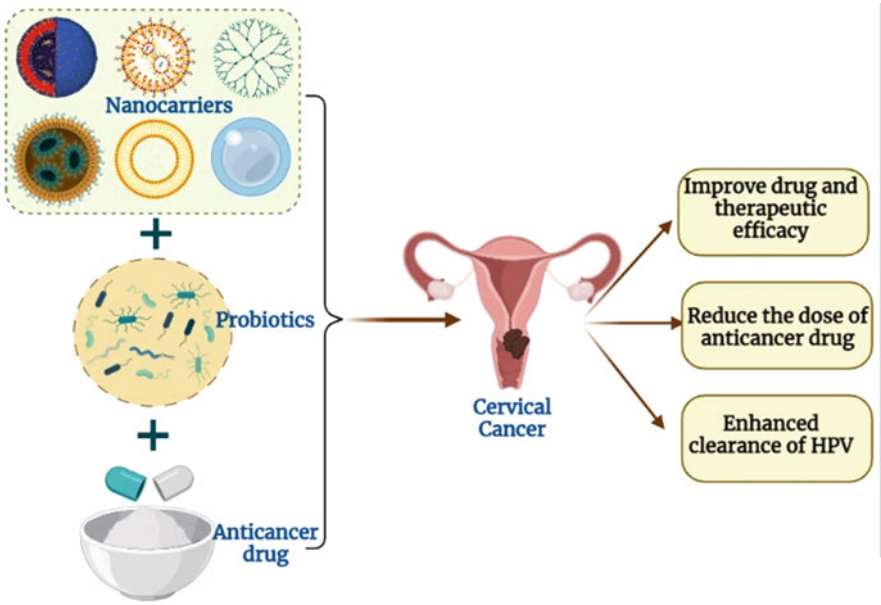

Fig. 6.4 Anticancer loaded nanocarriers and probiotics for the management of cervical cancer

6.18 Probiotics Provide a Better Diagnosis and Therapeutic Targets for Cervical Cancer

Because of engineering approaches that enable bacteria to identify a precise chemical in individuals/sufferers or generate an indication as a consequence, bacteria can be employed as a diagnostic probe (Zhou et al. 2020). Danino et al. (2015), for example, established an orally delivered probiotic-based diagnosis. They demonstrated that creating signals detectable in urine utilize *E. coli*; "Nissle 1917 is a non-invasive method" for detecting liver metastases. *Vibrio cholerae* produces a chemical that *L. lactis* has been used to detect (Mao et al. 2018). The color of the host's feces changes as the bacterium develops a diagnostic circuit, providing an early warning of cholera infection. As a consequence, engineering enable it feasible to employ probiotics as a tool for diagnosing cancer of cervical. Two probiotic strains, "*Lactobacillus reuteri* RC-14 including *Lactobacillus rhamnosus* GR-1," exhibited to assist with gynecological difficulties (Chew et al. 2015; Kohler et al. 2012). A series of research, the utilization of anti-infective medications such "*Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14" lowers the frequency of cervical cancer cases that are both falsely positive and falsely negative. Cytogenetic diagnoses are thus more certain in this process (Perisic et al. 2011). Investigators explored into the impact of probiotic strains on the quality of cervical smears involving the elimination of genital high-risk human papillomavirus

(Ou et al. 2019). "*Lactobacillus rhamnosus* GR-1 including *Lactobacillus reuteri* RC-14" were exhibited to have a substantial impact on the incidence of unsatisfactory with slightly abnormal cervical smears. However, they had no effect on HPV clearance. MiR-29a with miR-21 have been identified as the most often downregulated including elevated miRNAs implicated in the advancement of invasive cervical carcinoma, respectively (Pardini et al. 2018). MiR-20b, miR-9, miR-10a, miR-106 including miR-16 are some of the additional dysregulated miRNAs in cervical cancer. Furthermore, exfoliated cervix cells with dysregulated miR-34a, miR-375 including miR-125 are linked to cancer development. TLR-4, miR200b, with miR-21, all of which are connected to apoptotic induction, are found to be downregulated by vaginal-isolated *Lactobacillus lactis* (Rahbar Saadat et al. 2019). In addition, the probiotic *Escherichia coli* Nissle 1917 has been shown to restore the expression altitudes of certain miRNAs "(i.e., miR-143, miR-155, and miR-375)" (Rodriguez-Nogales et al. 2018). The development of NF-κB is triggered by HPV infection, and it impacts both innate involving adaptive immune responses. By downregulating NF-κB, the virus disables the immune system's inhibitory function, resulting in an infection that persists (Tilborghs et al. 2017). By blocking numerous signaling pathways, including the nuclear factor-B (NF-B) pathway, probiotics help to reduce inflammation. Furthermore, probiotics can inhibit the binding of lipopolysaccharides to the CD14 receptor, resulting in a reduction in total NF-κB activation and the generation of pro-inflammatory cytokines (Yousefi et al. 2019). NF-κB activation is also inhibited by *Lactobacillus fermentum*, *Lactobacillus johnsonii*, *Lactobacillus plantarum*, *Lactobacillus delbruekii*, including *Bifidobacterium longum* (Lee et al. 2018; Kim et al. 2018). Component with probiotics VSL#3 inhibited colitis-related carcinogenesis, and it was discovered that VSL#3 also suppressed the IL-6/STAT3 pathway (Do et al. 2016). "Exo-polysaccharides from *Lactobacillus plantarum* NCU116" are thought to be the catalyst for STAT3's adhesion to the ZO-1-occluding promoter region, according to Zhou et al. Additionally, therapy with this exo-polysaccharide reduces STAT3 expression, which in turn suppresses the production of occludin and ZO-1 (Zhou et al. 2018).

6.19 Future Direction

From various laboratory research it was found promising positive outcomes, which support the anticancer response of probiotics against cervical cancer. Thereafter, the current research studies reported the efficacy of synbiotics only for the promising prevention of cancer or as adjuvant therapy at the time of chemotherapy. A number of clinical trial studies are less to establish the potential impact of synbiotics in this purpose. Thus research on the anti-neoplastic function and mechanism of action of probiotics must be investigated (mainly at the time of therapy). Therefore, placebo-controlled clinical trial, double-blind, with a randomized must be done to gain a grant from the medical community and validate the promising efficacy of synbiotics as another approach for cancer management. The mechanism of probiotic use with

their products to preserve individual's flora is the next issue after finding the most beneficial flora for cancer prophylaxis including management. The main objective is to identify certain variants or groups of bacteria that might lessen the side effects of chemotherapy treatment.

6.20 Conclusion

To summarize, even though this subject is still in its adolescence, synbiotics have a substantial influence on the therapy and preventative measures of numerous forms of tumors. According to several well-established pathways, probiotics are effective in preventing malignancy. There are numerous drawbacks, including the scarcity of authorized clinical trials that are small, diverse, and frequently susceptible to major biases (Hassan et al. 2018). It is challenging to conclude that balanced eating, rich in fiber and moderate in meat and fish, has a protective impact against cancer that is greater than that. However, many Westerners must not eat this and have additional cancer and breast cancer, including diabetes and obesity. Even though the cost of lengthy intake would not have been negligible, the danger of employing synbiotics seems small. Finding any benefits in cancer incidence requires doing prospective longitudinal cohort investigations. Improved effectiveness is seen when using synbiotics as supplemental cancer treatments.

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Synbiotics in Gastric Cancer

7

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7.1 Introduction

After colon and lung cancer, gastric cancer (GC) is the third leading mortality cause, contributing for 782,685 mortality globally each year (Javanmard et al. 2018). Infection with *Helicobacter pylori*, age, a high sodium intake, consumption of alcohol, and cigarettes, and a low vegetable and fruit intake are all factors (Scott et al. 2015). Infection because of *Helicobacter pylori* is widespread, impacting more than half of the global populace, with a greater proportion in poor nations (Wan and El-Nezami 2018). The initial sign of *H. pylori*, which induces persistent inflammation, is chronic gastritis. The development of GC could be caused by damage to epithelial cells of the intestine as the illness advances (Kidane 2018). Microbiomes are microbiological colonies composed of bacteria, viruses, and fungus which thrive in different environments inside the body (Human Microbiome Project Consortium 2012). Since it includes the greatest variety of bacteria, the colon is one of the most extensively researched human microbial ecosystems (Villéger et al. 2019). Accordingly to maintain homeostasis and progression of the disease, the microbiome or its by-products perform pathologic and physiologic roles (Gilbert et al. 2018). The association that is between the human microbiome and illnesses has piqued researchers' curiosity in recent decades. Despite indications suggesting altering the

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microbiome-host ratio inside the gut may speed up the development of GC, the mode of action remains unclear. This book chapter provides an update on stomach cancer caused by *H. pylori* with the benefits of synbiotics for the treatment of gastric cancer. A thorough exploration of data from clinical trials using synbiotics to prevent or cure stomach cancer has also been discussed.

7.1.1 The Human Gastric Microbiome

For its acidic medium, the gastrointestinal was formerly thought to become a sterile organ. Marshall and Warren's discovery of *H. pylori* present in the abdomen of a person having peptic ulcers and gastritis, however, disproved this theory (Marshall and Warren 1984). Microbiologic procedures like identification, isolation, and culture were formerly used to examine the human stomach microbiome. However, most bacteria cannot be detected using this method because minor amount of stomach germs could be cultivated under conventional culture conditions. The bacteria like *Clostridium, Lactobacillus*, and *Veillonella* spp. employing culture-dependent approaches, they are by far the most often obtained from stomach (Zilberstein et al. 2007). In order to confirm the efficacy of synbiotics in the treatment of cancer, further clinical trials are required. In addition, contemporary techniques such as next-generation sequencing, random shotgun sequencing method and microarrays, have identified a vast number of species alternative to meals. The concentration of bacteria in the abdomen is around 10^2–10^4 CFU/mL, which is significantly less than that of the intestine (10^{10}–10^{12} CFU/mL) (Delgado et al. 2013). Under normal conditions, the most abundant phyla inside the stomach mucosa are *Firmicutes, Proteobacteria*, *Bacteroidetes, Fusobacteria, and Actinobacteria* (Delgado et al. 2013; Bik et al. 2006; Liu et al. 2019). The bacterial community of human digestive juices varies from the gastric mucosa; the first is controlled by *Bacteroidetes*, *Actinobacteria*, and *Firmicutes,* while the final is controlled by *Firmicutes and Proteobacteria* (Bik et al. 2006; Nardone et al. 2017; Sung et al. 2016). *Clostridium*, *Lactobacillus*, and *Veillonella*, bacteria prevalent inside the mouth and duodenum, may invade the abdominal part quickly (Zilberstein et al. 2007; Nardone et al. 2017). A lack of complete gut microbiota of digestive fluids may have been detected as a result of these findings. The precise mechanisms that cause inter-individual variations in the makeup of the stomach microflora are unknown. Numerous factors influence microbiome composition, including child-birth method, age, gender, nutrition, environment, location, culture, chemotherapeutic agents, histamine H2 receptor antagonist, proton pump inhibitors (PPI), and also the prevalence of *H. pylori* (Bokulich et al. 2016; Tsuda et al. 2015; Lloyd-Price et al. 2016; Haro et al. 2016). The acidic environment of a normal gut helps to prevent bacterial growth and infections (Howden and Hunt 1987). Continuing usage of a proton pump inhibitor (PPI) or an H2 antagonist lowers acid reflux secretion, resulting in bacterial growth (Alarcón et al. 2017). Using antibiotics, pH more than 4, and immunosuppression inside the gut are all associated with lower bacterial diversity (Von Rosenvinge et al. 2013). One of the research studies accomplished on

the stomach microbiota of identical twins revealed that genetic makeup had no effect on the establishment of gut bacterial populations; similar results were obtained for numerous other human body compartments (Dong et al. 2017; Lee et al. 2011).

7.1.2 Gastric Cancer

Despite the fact that the incidence of GC has reduced in recent times, the five-year rate of survival is less than 25%, with regional variations (Rawla and Barsouk 2019). The majority of gastric cancer and probiotics research has focused on eliminating the *H. pylori*, which is among the biggest sources of stomach cancer (Qureshi et al. 2019). *H. pylori* is one of Gram-negative bacteria which can invade the gut epithelium and cause serious damage to the acid-alkaline barrier. It is detected in individuals with peptic ulcers, chronic gastritis, and gastric cancer (Amieva and El-Omar 2008). In animal models including *L. acidophilus, B. bifidum, L. salivarius*, *L. rhamnosus,* and other probiotic strains, probiotics have been shown to inhibit *H. pylori* contamination (Patel et al. 2014). The efficiency of *H. pylori* elimination treatments involving a PPI and two antibiotics (clarithromycin with metronidazole or amoxicillin) has dropped in the latest days due to the growth of resistant strains of *H. pylori* (Graham and Fischbach 2010). As per a recent meta-analysis, supplementing antibiotic treatment with probiotics is particularly effective in eradicating *H. pylori*. Summarizes the findings of clinical trials examining the efficacy of probiotic microflora in combination with antibiotic treatment in eradicating *H. pylori* colonization (Li et al. 2015). According to the findings of this research, probiotic intake following *H. pylori* removal antibiotic treatment decreases adverse reactions, leading to increased compliance and, in certain situations, improved eradication ratios (Lü et al. 2016). Furthermore, gastric tumors that promoted lymphoid tissue development vanished following effective eradication. One of the postulated explanations for probiotic therapy is that these microorganisms can be present in the stomach and even remain there for a short period, enhancing the immune response and decreasing the occurrence of *H. pylori* inflammatory reactions on the stomach mucosal layer of the host (Russo et al. 2014).

7.2 Mechanisms of Gastric Carcinogenesis

7.2.1 *Helicobacter pylori* and Gastric Carcinogenesis

Gastric microbiota uses mechanisms such as inflammation, immune response modulation, tumor development and angiogenesis control, microbial metabolite synthesis, and DNA damage induction to promote gastric cancer. *Helicobacter pylori* is a spirally coiled Gram-negative bacteria that generates the enzymes like catalase, urease, and oxidase. *H. pylori* is estimated to infect humans around 50 million years ago and developed to flourish in the stomach's severely acidic pH. The flagellum as well as the spiral structure of *Helicobacter pylori* enables it to penetrate

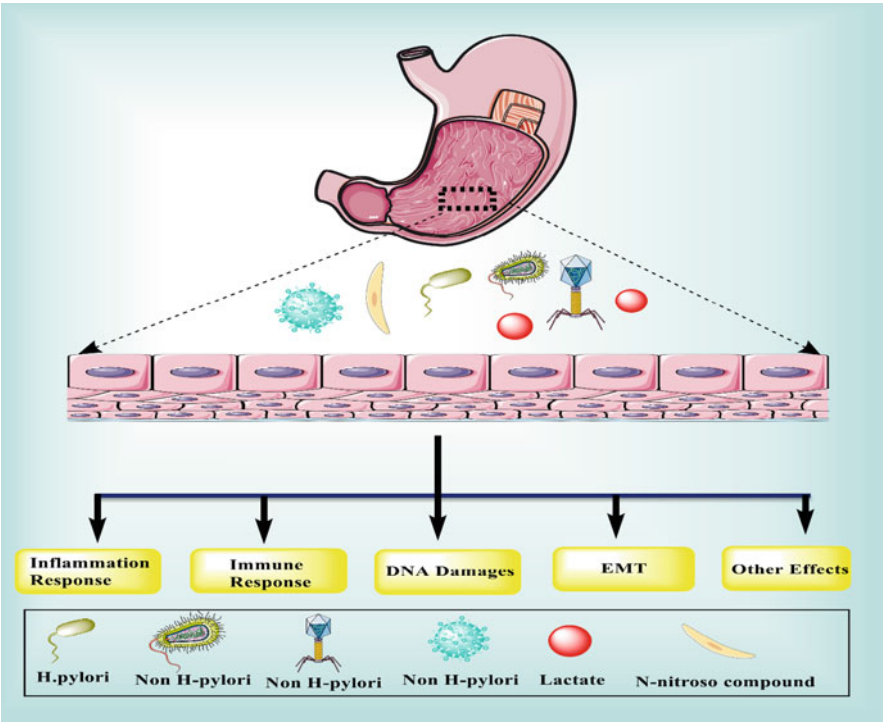

Fig. 7.1 The influence of microorganisms besides *H. pylori* upon the development of stomach cancer. The depletion of acid-secreting parietal cells is promoted by the inflammatory process produced by *H. pylori*, resulting in higher in gastric pH. Gastric microbiome dysbiosis primarily affected by differences inside the stomach's acidic condition, which allows other microorganisms to proliferate. Non-*H. pylori* organisms cause stomach cancer by their characteristics and microbial molecules, such as N-nitroso and salts of lactic acid. Creation of an inflammatory response, modulation of the immune response, induction of DNA damage, and stimulation of the EMT (epithelial-mesenchymal transition) are all viable techniques

the abdominal membrane and produce a systemic infection (Gu 2017; Sycuro et al. 2012). As urea is converted into ammonia by *H. pylori*'s urease, the acidity in the stomach can be controlled (Scott et al. 2010). For *H. pylori* to progress in the stomach, to escape from host's immune responses, and cause pathogenesis, it has to be able to traverse the gastrointestinal mucus, establish a secure zone near the gastric mucosal layer surface, and distribute its products to the host tissues (Zhang et al. 2017; Olofsson et al. 2014; Pachathundikandi et al. 2019). Genetic diversity owing to point mutations as well as inter- or intragenomic recombination has been tied to pathogenicity and proven to influence the probability of cancer transformation in *H. pylori* strains (Yadegar et al. 2019) (Fig. 7.1).

7.2.2 *Helicobacter pylori* Virulence Factors

Various virulence variables in *H. pylori* strains affect the probability of GC growth either indirectly or directly (Ansari and Yamaoka 2019). VacA is a significant virulence driver which is first found because of its ability to promote vacuolation in epithelia. VacA is a multipurpose toxin that has a range of impacts on numerous host types of cells (e.g., cells displaying antigens, phagocytic cells, gastric epithelial cells, mast cells, and T cells). VacA causes mortality in host epithelial cells by changing endocytic migration and enhancing mitochondrial membrane fluidity. VacA also regulates the specific immune system by restricting immune cell growth and proliferation and encouraging mast cells to express pro-inflammatory cytokines (e.g., IL-6 and TNF-α) to enhance *H. pylori*-associated gastritis and peptic ulcer disease. CagA, which is expressed by cagA genes present around one extremity of a cag pathogenicity island (cag PAI), is another virulence component linked to the advancement of GC (Hatakeyama 2017). CagA from *H. pylori* is delivered to host cells via the inner and outer surface of the cellular membrane which further transcribes the type IV bacterial secretion system (T4SS) (Chung et al. 2019). CagA could promote malignancy in intestinal cells by generating inflammation, promoting growth, suppressing apoptosis, changing cell–cell connections, and inducing cell polarity disruption after translocation (Buti et al. 2011; Bagnoli et al. 2005; Yang et al. 2018).

7.2.3 *Helicobacter pylori* and Immunological Response

After infection with *H. pylori*, an adaptive or innate immune response is elicited (de Melo et al. 2014; Bimczok et al. 2010). Host defenses are strengthened by *H. pylori*'s virulence factors. PAMPs (pathogen-associated molecular patterns) of the *H. pylori* bacteria are recognized by host cells' pattern recognition receptors (PRRs), which triggers the innate immune response. TLRs bind to lipoteichoic acid, lipoproteins, lipopolysaccharides, two-stranded RNA, flagellum, demethylated nucleic acid bases, particularly *H. pylori* CpG repeated sequences (Satoh and Akira 2016). TLRs activate NF-kB, AP-1, and the interferon regulatory factor (IRF) when they detect PAMPs, leading to the generation of inflammatory mediators such interferon (IFN), interleukins 2, 6, 8, 12, and TNF-α (Kawasaki and Kawai 2014; Nejati et al. 2018). While *H. pylori* may be able to evade the host's innate immune system as it is resistive to PRRs, it may also be able to survive for longer periods of time (Devi et al. 2015; Sun et al. 2013). In adaptive immune system, CD4+ T cells are critical mediators of the host immunological response to *H. pylori* infection (Karkhah et al. 2019). While CD4+ T cells were more common in GC tissues than the normal ones, CD8+ T cells were seen in the opposite manner (Huang et al. 2014). Virulence factors from *H. pylori* trigger Th1 and Th17 cell responses, resulting in increased production of IFN α, interleukin-17 and TNF-α during the innate immune responses (Bimczok et al. 2010). As a result, *H. pylori*-infected patients have activated Th1 or Th17 cells, leading to inflammatory reactions

(Beigier-Bompadre et al. 2011; Bimczok et al. 2010). While *H. pylori* infection rates along with acidity of abdomen were reduced as a result of inflammatory responses, while elevating invasion by other microorganisms (Pereira-Marques et al. 2019). RNS (reactive oxygen and nitrogen species) are produced in response to *H. pylori* infection and chronic inflammation, which causes DNA damage including double-strand DNA breaks and point mutations, disrupts signal transduction pathways, and trigger cell death or apoptosis in intestinal cells. In *H. pylori*-positive epithelial cells, the DNA repair pathway was compromised (Han et al. 2020). By infecting cells and creating genetic instability, *H. pylori* can cause gastric cancer. ROS also permits *H. pylori* to alter its DNA, letting it to adjust according to the environment around (Gobert and Wilson 2017) (Fig. 7.1). *H. pylori*, on the other hand, inhibits the immune system of the host in contrast to its action on effector T cells. Tregs are immune system cells that prevent potentially damaging aberrant or hyperactive immune responses (Liu et al. 2015). Foxp3 (Forkhead box proteins) and Treg cells (T regulatory) are the most crucial immune-suppressive regulators (Deng et al. 2010). In both clinical and preclinical trials, Foxp3 polymorphisms are related to incapacitating autoimmune diseases (Colobran et al. 2016). Elevated expression of Foxp3 was found in tumor-infiltrating cells and the proportion of Foxp3+ Tregs was higher in both malignant and peritumoral samples (Yang et al. 2021). T cell proliferation was also suppressed and associated with a phase of tumor node metastasis in GC patients who had Tregs in their system. Immune responses of CD4+ memory T cells from *H. pylori*-positive people against *H. pylori* antigens were improved by Foxp3 along with Treg cell reduction (Lundgren et al. 2003). Treg-depleted *H. pylori*-infected animals showed a significant amount of stomach inflammation and decreased *H. pylori* growth (Rad et al. 2006). There are two kinds of Foxp3+ Tregs: those with ICOS expression and those with ICOS+ (Ito et al. 2008). Patients with GC who had ICOS+ Foxp3+ Tregs released IL-10 and TGF-α, which inhibited T cell and DC function, and hence were connected to poor clinical trials (Ito et al. 2008). As a consequence, new evidence suggests that Tregs suppress host immunological response, exacerbating *H. pylori*-induced aggravation and accelerating the onset of GCDCs serve as a connection between both the adaptive and innate immune systems. Immunological activity (increasing immunity or encouraging immunological tolerance) and cancer clinical factors are affected by DC maturity status (Karthaus et al. 2012). DCs which induce immunologic resistance and tumor growth are known as plasmacytoid (p). The increased occurrence of plasma DC antigen (BDCA)2+ pDCs in peritumoral and tumor samples indicates a poorer prognosis for GC patients (Ling et al. 2019). Tumor-infiltrating pDCs can cause immunosuppression by stimulating the growth and activation of ICOS, Foxp3, and Treg cells (Conrad et al. 2012).

7.2.4 Non-*H. pylori* Bacteria That Promote Gastric Carcinogenesis

Gut microbiome patterns in GC patients of Korea showed a larger proportion of *P. copri* and *P. acnes* than in control subjects, indicating that the presence of these

microorganisms increased the likelihood of GC (Gunathilake et al. 2019). Predominant lymphocytic gastritis is caused by *P. acnes* and its components stimulating the natural killer 2D pathway and creating pro-inflammatory cytokines, which in turn stimulates the NKG2D pathway (Montalban-Arques et al. 2016). Interleukin 15 (IL15) has been linked to cancer formation through the NKG2D–NKG2DL pathway and NKG2D ligand (NKG2DL) levels (Oppenheim et al. 2005). In contrast, *H. pylori* appears incapable of activating the NKG2D–NKGDL pathway or inducing the synthesis of IL15 (Montalban-Arques et al. 2016; Gálvez et al. 2020). In the human microbiome, Prevotella has always been the most frequent bacterial species. *P. copri* enhances the host's resistance to ROS and produces the pro-inflammatory redox protein thioredoxin under a range of circumstances (Hofer 2014). Although the number of *P. acnes* in the GC tissue of 276 Chinese individuals increased, the number of *P. copri* dropped (Liu et al. 2019). Even if more study is necessary to determine the involvement of *P. copri* in cancer development, these are all contradicting results.

The microbiota in the gut and the microbiome in the stomach are both thought to have an impact on the immune system of the body. Peritumoral and tumoral tissues had higher concentrations of BDCA2+ pDCs and Foxp3+ Tregs than normal tissues. In a study, it was disclosed that BDCA2+ pDCs and Foxp3+ Tregs were positively linked with the quantity of *Stenotrophomonas* and *Selenomonas*, while *Comamonas* and *Gaiella* were adversely associated with these variables (Ling et al. 2019). In addition, the prevalence of *Stenotrophomonas* and *Selenomonas* and was positively associated with the population of BDCA2+ pDCs and Foxp3+ Tregs, whereas the prevalence of *Gaiella* and *Comamonas* was inversely associated with the number of BDCA2+ pDCs and Foxp3+ Tregs (Ling et al. 2019). In addition, studies comparing healthy individuals to those with GC revealed that the *Comamonas* count was higher in the healthy individuals (Dong et al. 2019). Changes in the microbiome patterns of the gut may affect the number of immune cells, thereby generating an immunosuppressive environment. It has been discovered that Tregs and pDCs suppress antitumor immunity, allowing tumor cells to evade immune surveillance (Huang et al. 2014). While *Stenotrophomonas* managed to avoid phagocytosis and stimulated DCs to produce TNF- and IL-12 in order to promote inflammation, nothing else is available about its interactions with DCs in humans (Roscetto et al. 2015). To fully comprehend how well the microbiome affects immune modulation, more research should be done.

The presence of elevated *Fusobacterium* in GC patients is predictive (Hsieh et al. 2018). Due to *Fusobacterium nucleatum*, Lauren's diffuse-type GC had an even worse prognosis than her intestinal GC (Boehm et al. 2020). In addition, contamination with *Fusobacterium sp*. has been linked to p53 expression as well as tumor-infiltrating lymphocytes in GC tissues (Nie et al. 2021). Colorectal cancer, appendicitis, inflammatory bowel illness, and pancreatic cancer have been associated with *F. nucleautum* (Swidsinski et al. 2011; Shaw et al. 2016; Del Castillo et al. 2019). In contrast, the pathogenesis of *F. nucleatum* in GC is still unidentified (Rubinstein et al. 2013). In cancer cells, contact among E-cadherin of epithelial tissue and F. nucleatum adhesin FadA promoted β-catenin as well as the

signaling cascade incorporating Wnt. *F. nucleatum* directly stimulates NF-B (Kostic et al. 2013), boosting the production of pro-inflammatory cytokines including interleukins 6 and 8 including TNF. Additional *F. nucleatum* adhesin, fibroblast activation protein 2, can decrease anti-tumor immunological activity by binding to the T cell immunoreceptor tyrosine-based inhibitory motif domain (TIGIT) receptor (Gur et al. 2015) and immunoglobulin produced by NK cells. *F. nucleatum* could use a standardized technique to enhance the rate of gastric carcinoma (Fig. 7.1).

7.3 Role of Synbiotics in the Treatment and Diagnosis of Gastric Cancers

Traditional therapies employed for GC, including radiation therapy, chemotherapy, and surgery, proved ineffective. Novel treatment options are therefore required. Although microorganisms were once thought to be carcinogenic, there is no evidence that they have anticancer effects. The importance of the microbiome in cancer treatment was first hypothesized in 1867, when Streptococcus pyogenes infection resulted in a patient's cancer remission (Sawant et al. 2020). Microorganisms have anticancer properties in various ways like (1) invading tumors, (2) releasing chemicals, (3) decreasing nutrients essential for tumor development and metabolism, (4) functioning as a delivery channel for anticancer medications, (5) building biofilms, (6) improving host defense. HPRP-A1, 15-mercapto positively charged peptides, and HPRP-A2 derivatized from the nitrogen terminal of *H. pylori* ribosomal protein L1 (Mai et al. 2015). Both HPRP-A2 and A1 possess antibacterial and anticarcinogenic activities. HPRP-A1 occurs as a peptide including membrane and further disrupts the membranes of group of tumor cells (Zhao et al. 2015b), making it a common drug delivery system for cancerous cells. Especially the KLA peptide promotes apoptotic cell death by disrupting the mitochondrial membrane (Hu et al. 2018); however, it exhibits lower membrane infiltration. HPRP-A1 encourages the entrance of KLA peptides into malignant cells (Hao et al. 2019), where it binds to membrane of mitochondria and induces the mortality of tumor cells. HPRP-A2 causes GC cells to die by increased ROS generation, activating caspase-8, caspase-9, and caspase-3, lowering the potential of the mitochondrial membrane and stopping the cellular cycle in the G1 stage (Zhao et al. 2015a). Ieodoglucomides B is a glycolipopeptide generated by *Bacillus licheniformis* that, along with both HPRP-A1 and HPRP-A2 have a lethal effect on lines of intestinal cancer cells. FW523–3 seems to be a molecule of lipopeptidic nature produced from the cultural broth of the bacteria found in marine. *Micromonospora chalcea* that inhibits the growth and maturation of numerous types of malignant cells, including GC cells (Tareq et al. 2012; Xie et al. 2011).

7.3.1 Clinical Implication

CagA and H. VacA are two virulent that have a direct impact on malignancy in *H. pylori*. VacA, a multimeric pore-forming protein with a large molecular mass, exist in entire *H. pylori* variants, as well as its preservation in gastrointestinal system is simplified by pore creation across epithelial layer (consequently urea outflows that allows *H. pylori* to accelerate hydrolytic degradation of urea as a method of shielding to counteract stomach acidity), and also inhibition of T cells and macrophages (Wroblewski and Peek 2013). CagA which comes under the category of strain-specific protein, inhibits the apoptotic pathway in epithelial cells, resulting in morphological abnormalities such as cell dispersion and extension, as well as disruption of cell polarity (Chen et al. 2016). *H. pylori* implicitly promotes cancer through influencing the components present in the microbiota of the gut (Weng et al. 2019). During the century of culture-based research, the gut was believed to be sterile in healthy individuals; however, advancements in DNA nucleotide sequences of invariant rRNA revealed that the stomach has a complex microbiota, composed primarily of phylum bacteria. Bacteria are categorized as Proteobacteria, Actinobacteria, Firmicutes, Fusobacteria, and Bacteroides (Frank and Pace 2008).

H. pylori-induced gastritis could be either corpus or antral-dominated. *H. pylori*-mediated enhanced gastrin secretion causes higher stomach acid production in antral-predominant gastritis, making people more sensitive to duodenal ulcers while protecting them from GC (McColl et al. 1998). *H. pylori* reduces production of acid via inflammatory mediators in corpus-dominated gastritis, later, atrophic gastritis results from the slow degeneration of gastric glands. Microbes that would ordinarily be killed by the harsh gastric environment can survive because gastric acid secretion is reduced (Weng et al. 2019). It is unclear how the changed microbiota interacts with *H. pylori* to cause cancer. According to one theory, such microbes (nitrosating bacteria) could convert nitrogen molecules in gastric juice into potentially carcinogenic N-nitroso compounds (NOCs) (Louis et al. 2014). NOCs can be produced by a variety of bacteria, like *Lactobacillus, E. coli, Veillonella*, *Haemophilus, Nitrospirae, Clostridium, Streptococcus, Staphylococcus, Neisseria commensals* and *Prevotella* of the oral cavity, as well as these, are connected with a reduced probability of GC formation (Deo and Deshmukh 2019). Researchers of microbiota are interested in determining which species predominate in the intestinal mucosal wall of GC patients. Investigators were using the INS-GAS experimental models on mice (insulin activator attempting to control the up-regulation of gastrin) for demonstrating that *H. pylori* infection in conjunction with intestinal flora colonization increases the likelihood of gastrointestinal intraepithelial neoplasia (GIN), emphasizing the importance of gastrointestinal bacterial species for the expansion of GC (Zhang and Moss 2012). In addition, they discovered that *H. pylori* mice colonized with limited Altered Schaedler's flora (ASF) (3 species) owes the same risk of developing GIN as mice colonized with eight species, indicating that intestinal infections do not influence carcinogenesis (Lertpiriyapong et al. 2014). Because a substantial portion of the stomach microbiota cannot be cultivated, human studies are limited. DNA sequencing has improved. Although earlier research had mixed

results due to reduced sample sizes, the following investigations discovered substantial changes in terms of bacterial population, structure, and load among individuals with GC and/or HP and subjects (Weng et al. 2019). Patients with GC had a different microbial makeup than controls, which was referred to as dysbiosis. However, no consensus has yet been achieved on which bacteria the most prevalent and likely are involved in malignancy (Zhang et al. 2020). The amount of nitrosating microorganisms in patients lacking *H. pylori* was two times higher as in other three categories, but just not substantially so, reinforcing the notion that NOCs have a crucial function in cancer progression (Lam 2021). Wang et al. revealed five bacterium species with cancer-promoting properties. *Escherichia coli, Lactobacillus, Nitrospirae, Lachnospiraceae,* and *Burkholderia fungorum* strains of bacteria were found abundantly in GC patients; *Nitrospirae* bacterium were recovered from all GC patients, but not chronic gastritis patients (Nasr et al. 2020). However, these results were inconsistent among studies. In contrast to those with intestinal metaplasia or atrophic gastritis, persons with GC have a significantly greater quantity of oral bacteria than that by either atrophic gastritis or intestinal metaplasia. Network research revealed that *Peptostreptococcus stomatis*, *Parvimonas Micra*, *Slackia exigua*, *Dialister pneumosintes*, and *Streptococcus anginosus* were the most significant (Ruiz et al. 2019). To screen for GC, an oral microbiota detection score approach with a 97 percent sensitivity and 7.7 percent false-positive rate was developed (Zeller et al. 2014). However, researchers projected the changes in stomach commensal flora, rather than a particular pathogen, are important in gastric carcinogenesis (Noto and Peek Jr 2017). There is no agreement on the diversity of microbiota and found a more diverse microbiome in GC patients although other research found the contrary. This is assumed that variables affecting intestinal flora like age, gender, ethnicity, and *H. pylori* infection, contributed to the disparity. Researchers hypothesized that decreased bacterial diversity could also be linked to the development of GC because decreasing bacterial diversity was linked to the development of CRC (Goecke and Imhoff 2016).

7.3.2 Mechanism of Action of Synbiotics

7.3.2.1 Antioxidant and Anti-inflammatory Actions

Kefir supplementation appears to reduce the consequences of excessive ROS, according to a growing body of evidence, and has been recommended as a potential curative and preventive therapy for a variety of chronic illnesses (Vasquez et al. 2019). It has been proven that Kefir aids with cardio-metabolic disorders, neurological diseases, and pancreatitis in recent studies. Kefir's biological resistance towards the ROS production that also happens when human physiology's antioxidant limit is reached, due to oxidative stress such as through numerous irreversible cellular injury or oxidization of lipids, proteins, nucleic acids, and carbohydrates might be a key mode of action (Pimenta et al. 2018). The production of ROS is related to electron transport mechanisms and enzymatic processes in mitochondria (e.g., xanthine oxidase and NADPH oxidase). Kefir extracts has already been demonstrated to

enhance the efficiency of ROS scavenging enzyme such as superoxide dismutase, and catalase and its constituents have now been proven to actively scavenging intracellular ROS (Nagira et al. 2002). Moreover, research findings have shown that kefir could decrease oxidation process and increase antioxidant glutathione (GSH) in an animal model of carcinogen-induced colon cancer, implying that increasing NO levels activates the adaptive/innate immune response, confirming previous findings that kefiran enhances the immune reaction and implying that biogenic compound modulation takes place first in intestine then in the liver (Bozkurt et al. 2020). Both in in vitro and animal experiments, it was later discovered that consuming kefir elevates the concentrations of TNF, and INF cytokines interleukin (Zubiría et al. 2017). The majority of such cytokines, on either hand, promptly returned to baseline after few days of kefir use, but the anti-inflammatory cytokines were chronically elevated. Kefir tends to lower pro-inflammatory cytokines while raising anti-inflammatory IL-10 (Smoak 2019). Several analytical studies employing kefir as well as fractions and isolated-kefir microbes demonstrate that anti-inflammatory cytokines promote the Th2 response while decreasing the pro-inflammatory Th1 response (de Lima Barros et al. 2021).

7.4 Conclusion

To summarize, pre-, pro-, and synbiotics have considerable potential for tumor prevention and therapy; however, the research is still in its early stages. Although many established pathways via which probiotics may also have positive benefits, clinical research are limited. Use of prebiotics, probiotics, and synbiotics as cancer adjuvants has a stronger evidential basis. Probiotics have anti-mutagenic properties, anticarcinogenic effects, alteration of differentiation process in cancer cells, development of short-chain fatty acids, modification of tumor expression levels, activation of host immune response, inhibition of microbes which convert pro-carcinogens to carcinogens, modification of colonic motility, as well as other positive effects on gastrointestinal cancers, according to numerous animal and in vitro studies models. However, this line of research is intriguing and fascinating, we are still many generations away in figuring out how to use these compounds, and its final significance may be restricted. Therefore, this is safe to infer that probiotics, prebiotics, and synbiotics have a lot of promises as just a new approach for stomach cancer prevention. Such favorable effects may be the result of a variety of mechanisms, most notable of which is change of intestinal micro biota, that influences host immunity and metabolism. However, there is a lack of suitable follow-up information from human's clinical research utilizing probiotics as cancer biotherapeutics. As a consequence, extensive human clinical trial research is required to find viable cancer strains, dosages, and delivery regimes for specific cancer kinds and phases as just new treatment strategy for gastric cancer therapy.

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Synbiotics in Hepatocellular Carcinoma

8

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8.1 Introduction

Foods that have been given new labels indicating specific benefits because of this line of study are known as functional foods (Webb 2011). An idea of "functional meals" underlines that, it plays a vital role for survival; other than this it also helps in preventing and lowering risk factors for various ailments and it also improves crucial physiological processes by receiving the sufficient number of vitamins, lipids, proteins, and other nutrients from functional foods. Since then, researchers have worked to understand how a variety of food nutrients and ingredients can improve health or preventing chronic diseases (Cencic and Chingwaru 2010).

8.1.1 Probiotics

Probiotics is defined as non-pathogenic, live, bacteria that, when taken in adequate quantities, benefit the host's health. In GIT small intestine to the colon, the human gastrointestinal tract (GIT) is home to a wide range of bacterial species,

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with 10^7–10^{12} cells per gram of intestinal material (Le Leu et al. 2010; Guarner and Malagelada 2003). The gut microbiota is largely consistent up until environmental influences, a person's lifestyle, and altered genetic makeup cause changes.

Some of the commonly used microorganism in probiotics include *Lactobacillus reuteri*, *bifidobacteria*, *Lactobacillus rhamnosus,* specific strains of *Bacillus coagulans, Lactobacillus casei*, *Escherichia coli strain Nissle, Lactobacillus acidophilus-groups* a limited enterococci, furthermost remarkably *Enterococcus faecium* (SF68) and the yeast *Saccharomyces boulardii*. By increasing the countenance of tight junction proteins, probiotic metabolites and probiotic cell components support the integrity of the gut epithelium. To preserve the integrity of the gut epithelium, probiotic bacteria's cell surface proteins can reduce inflammation of gut epithelial cells and suppress cell death. Additionally, probiotics' capacity to boost goblet cell mucus formation and the release of antimicrobial peptides shields the epithelium of gut from harmful bacteria (Liu et al. 2020).

8.1.1.1 Uses

To maintain health, the gut microbiota performs essential host activities such as immunological and nutritional status (Cerf-Bensussan and Gaboriau-Routhiau 2010).

1. One of the probiotics' well-documented effects is the prevention or shortening of the complaints and duration of antibiotic- or rotavirus-induced diarrhea as well as the relief of lactose intolerance-related complaints (Borchers et al. 2009).
2. Decreased levels of putrefactive (bacterial) metabolites and/or cancer-promoting enzymes in the stomach.
3. Elimination and prevention of vague and inconsistent stomach symptoms in healthy individuals.
4. Positive benefits on bacterial overgrowth, inflammation, and other problems related to inflammatory bowel illnesses, *Helicobacter pylori* infections, and other conditions.
5. Normalization of bowel movements and stool consistency in people with constipation or an agitated colon.
6. Treating or preventing atopic disorders and allergies in babies.
7. Treatment of urogenital infections as well as the prevention of infectious diseases like the common cold and influenza.

Regarding the prevention of cancer, therapy or prevention of (IHD) ischemic heart diseases, or the enrichment of autoimmune diseases (such as arthritis), they are insufficient or at best preliminary evidence (Weese 2002).

They have demonstrated benefits for reducing lactose intolerance, lowering blood cholesterol, preventing cancer, treating constipation, boosting immunity, controlling obesity, and treating vaginitis.

Probiotics, prebiotics, and synbiotics have been shown to have anti-cancerous properties against bladder, colorectal and breast cancer in clinical and pre-clinical trials (Feyisetan et al. 2012; Delzenne and Cani 2011; de LeBlanc and Perdigón 2010).

Some probiotics are believed to have an antioxidant effect as whole cells or lysates. They are also engaged in the synthesis of nutrients and improving the body's absorption of those nutrients.

Additionally, probiotics have demonstrated their inherent effects in lowering allergies, cancer, AIDS, and other infections of the urinary and respiratory tract system. There are various arbitrary data on their advantageous impact on fatigue, type II DM, autism, aging, fatigue osteoporosis, and obesity (Harish and Varghese 2006).

Endotoxemia, a key risk aspect for malignancies that endorses pathogenesis through prolonged hepatic inflammation, has been linked to altered gut microorganisms.

By limiting the translocation of GIT bacteria and their products of metabolites into the liver, bacteria of probiotic can also safeguard intestinal epithelial function and prevent bacterial endotoxemia (Wan and El-Nezami 2018). In *Lactobacillus* sp. and *Bifidobacterium species* in obese people, microorganism probiotics may recover fatty liver and insulin resistance (Hernández-Ceballos et al. 2021).

8.1.2 Prebiotics

Prebiotics are a class of indigestible foods that have an optimistic impact on the host by encouraging the development and activity of the gut microbiota.

Due to their capacity to modify inflammatory response, intestinal flora and intestinal permeability, prebiotics have been hypothesized to be likely helpful in delaying the advancement of disease and preventing the development of problems (Gibson and Roberfroid 1995).

Prebiotics have many beneficial effects, including regulating colonocyte function, enhancing electrolyte absorption and water, reducing intraluminal pH, suppressing pathogen growth, altering gut immunological homeostasis, and affecting inflammatory processes.

Galactoglucomannans, mannan oligosaccharides, inulin, lactose, and oligofructose are examples of prebiotics that are often employed and gastric acidity and digestive enzymes was not obstructed by these prebiotics; hence, prebiotics plays tremendous factor for our health (Slavin 2013). Three to ten sugar units from yeast and plant cell walls make up the short-chain carbohydrates utilized as prebiotics (Yoo and Kim 2016). Hemicellulose, cellulose (mannan, xyloglucan, beta-glucan, and xylan), and pectin make up polysaccharides. All these complex carbs influence the microbial communities in the gut favorably (Flint 2012).

Some herbs are used to treat inflammatory immune disorders that had prebiotic effects on the host. These medicinal herbs include *Zingiber officinale, Piper nigrum, Ocimum sanctum*. *Zingiber officinale* and *Ocimum sanctum* have been shown to have higher growth and prebiotic activity in *Lactobacillus* and *bifidobacteria* compared to the most frequently used prebiotic fructooligosaccharides (FOS). Nevertheless, *Piper nigrum* has similar prebiotic activity to the most essential used prebiotic FOS. These traditional herbs are used to regulate the gut flora and in the long run it

prevents systemic swelling and related disorders (Liu et al. 2017). For plant-based prebiotic foods, lotus seed-resistant starch (Zeng et al. 2018) and burdock root (Moro et al. 2018) are also important. Already reported studies had been conducted to investigate the effects of ingestion of prebiotics consisting of xylooligosaccharides, fructooligosaccharides, resistant dextrins, and polydextroses on the immunity and structure of the microbiotic gut in patients with perioperative colorectal cancer. (Christie and Andrea 2021; Gulzar et al. 2019).

8.1.3 Synbiotics

The term "synbiotics," coined by Gibson, is a synergistic product of probiotics and prebiotics that works together to improve a person's gut and overall health (Vrese and Schrezenmeir 2008). Synbiotics enhance the survival and transplantation of live microbial supplements in the GIT by selectively encouraging the growth of one or a limited number of beneficial bacteria and/or activating metabolism and have advantageous effect on the host. Synbiotics were advanced to overwhelm the survival difficulties of probiotics. The reason for using synbiotics seems to be based on observations showing improved survival of probiotic bacteria as they pass through the upper GIT tract. Additional well-organized implantation in the colon, also stimulating effects on probiotics and ubiquitous bacterial growth, contributes to intestinal homeostasis and maintenance of a healthy body (Peña 2007). A Probiotic strain used in synbiotics preparations includes *Lactobacillus, Bifidobacterium,* and *S. boulardii, B*. The main prebiotics used are xylose-oligosaccharides (XOS), GOS, fructooligosaccharides (FOS), inulin from natural sources, such as chicory and yacon roots, exists alike.

Synbiotics also alter the composition of the colonic microbial flora, lessen the inflammatory process of the intestinal mucosa, can persuade IBD remission, prevent traveler's diarrhea, and improve the patient's overall quality of life. (Pokusaeva et al. 2011). Data reported that, male rats with hypercholesterolemia were fed rice bran fermented with *L. acidophilus* demosntrated improvement in the health quality (Oberreuther-Moschner et al. 2004), synbiotics have also been shown to be promising for the regulation of lipid profiles. In a research article, it has been reported that the 24 hypercholesterolemia male pigs were given synbiotic preparations of *L. acidophilus* FOS, mannitol, ATCC4962, and inulin for 8 weeks and showed hopeful hypercholesterolemia activity (Liong et al. 2007). Symbiotics treatment prevented the suppression of azoxymethane-induced NK cell activity in Peyer's patches. This is an effect not observed with individual probiotic and prebiotic treatments (Saulnier et al. 2009).

Administration of dietary oligofructose, *B. longum,* and inulin inhibits the development of preneoplastic lesions and *B. longum* suppressed breast and colon cancer (Kaur and Gupta 2002). Synbiotics seem to be a very attractive suggestion for boosting immune function. Combining *Bacillus coagulans* with dietary inulin for 6 weeks significantly reduced C-reactive protein levels and amplified glutathione levels (Panda et al. 2006). Synbiotic supplementation with *bifidobacteria*,

Lactobacillus, and 10% FOS in rats fed a high-fat, low-fiber diet inhibited GI and systemic inflammation, and the effect was comparable to FOS supplementation (Delcenserie et al. 2008).

Treatment of AQ3HLA-B27 rats that are prone to inflammation with Lactobacillus, bifidobacteria, and 10% FOS improved histological changes due to inflammation (Erejuwa et al. 2014). Synbiotics seem to be a very attractive suggestion for boosting immune function. Combining *Bacillus coagulans* with dietary inulin for 6 weeks significantly reduced C-reactive protein levels and increased glutathione levels (Panda et al. 2006). According to animal studies, synbiotic or probiotic treatment improves liver function in rats with liver dysfunction without alcohol and maintains structural integrity of the intestinal barrier. Previous studies have shown that synbiotic supplements enhance beneficial bacteria, repair the gut flora, limit endotoxin migration through intestinal tight junctions, and improve alcohol-induced liver damage. It has been reported that synbiotic supplements reduce liver damage, improve intestinal health (including microbial flora permeability and composition), and decrease muscle wasting in rats chronically fed ethanol (Parnell et al. 2012). Synbiotic supplements reduced liver damage, intestinal health (including microbial flora permeability and composition), and muscle wasting in rats chronically fed ethanol. In addition, the association between intestinal health and muscle destruction was investigated when symbiotics were given to rats with ethanol-induced liver lesions (Soriano et al. 2013; Endo et al. 2013).

Sl no.	Symbiotic treatment	Effect observed	Reference
1	Rice bran fermented with *L. acidophilus*	Lowering of lipid profile	Oberreuther-Moschner et al. (2004)
2	mannitol, inulin *L. acidophilus* and ATCC4962, FOS	Hypercholesterolemic	Liong et al. (2007)
3	*Bifidobacterium lactis* enriched with oligofructose	Prevented azoxymethane-induced suppression of NK-cell activity	Saulnier et al. (2009)
4	*B. longum* and oligofructose and inulin	Suppressed mammary and colon cancer	Kaur and Gupta (2002)
5	*Lactobacillus*, *Bifidobacterium*, and 10% FOS	Improved the histological changes due to inflammation	Erejuwa et al. (2014)
6	*B. lactis* in synbiotic combination with resistant starch	Restoration of the number of CD8-positive T lymphocytes	Yamazaki et al. (2000)
7	*B. lactis* and *L. rhamnosus* with inulin enriched with oligofructose	Reduce the incidence of adenomas and cancers induced by azoxymethane	Femia et al. (2002)
8	*B. coagulans* with inulin	Reduction in the levels of C-reactive protein	Panda et al. (2006)
9	*Lactobacillus*, *Bifidobacterium*, and 10% FOS	Suppressed intestinal and systemic inflammation	Delcenserie et al. (2008)

(continued)

Sl no.	Symbiotic treatment	Effect observed	Reference
10	*Lactobacillus plantarum* LS/07 CCM7766 with prebiotic inulin	Alleviates the intestinal inflammation in rats exposed to *N*,*N*-dimethylhydrazine	Štofilová et al. (2015)

8.1.3.1 Mechanism

Fermentation of resistant starch and fiber caused by bacteria in the colon releases short-chain fatty acids (SCFA). The synthesis of SCFA by a small number of species/genera of gut microbiota such as *Lactobacillus* and *bifidobacteria* is known to regulate anti-inflammatory responses and regulate proliferation and cell differentiation (Tajiri and Shimizu 2017).

SCFAs such as acetates, propionates, and butyrates have many biological roles that affect the structure and function of microbial communities. Bacterial conversion of indigestible carbohydrates and fibers to SCFA provides additional energy to the host, as the alien microflora has an enhanced ability to remove energy from food, according to the energy yield hypothesis. Over time, it can lead to personal obesity. Similarly, G protein-coupled receptors (GPRs) can recognize SCFAs associated with lipid and glucose degradation. SCFA activates two major proteins, GPR43 and GPR41, that are expressed in adipocytes and invade endocrine L cells. By activating GPR, inflammation is reduced in the intestine, a hormone that helps regulate insulin secretion is mimicking glucagon-like peptide (GLP). Intestinal endocrine L cells express GLP and secrete the intestinal nutrition hormone GLP-2. This reduces the translocation of lipopolysaccharide (Den Besten et al. 2013).

8.1.4 Hepatocellular Carcinoma (HCC)

One of the primary cancer HCC is the leading source of cancer-related deaths worldwide. Extrinsic risk factors for developing HCC include chronic liver infections due to hepatitis B (HBV) and C (HCV) viruses, alcoholic and non-alcoholic liver disease (ALD and NALD), and non-alcoholic fatty liver disease. Includes liver disease (NAFLD) and steatohepatitis (NASH) (Kulik and El-Serag 2019). Genetic background affects the pathogenesis of HCC, and the confluence of germline mutations and single nucleotide polymorphisms (SNPs) is a built-in risk factor for the disease's prognosis. These risk factors increase the likelihood of liver damage and subsequent fibrosis leading to liver cirrhosis and HCC.

8.1.4.1 HCC Epidemiology and Causes

HCC is more observed in men than in women, with a global prevalence of 2.4 males to females. The most common announcement age is 30–50 years. China, Mongolia, Southeast Asia, and sub-Saharan Africa in western and eastern Africa are all home to HCC. Apart from Japan, Italy, and France, the prevalence of HCC is low in industrialized countries around the world. HCC has risk factors for a variety of

etiologies, some of which have been shown to be expressively related with the development of HCC. Hepatitis viruses such as hepatitis D virus (HDV), HCV and HBV are powerfully associated with the development of HCC. Therefore, the global distribution of HCC reflects the global distribution of viral infections. It also describes some additional risk factors.

About 80–90% of HCC cases are associated with cirrhosis. In addition, the presence of multiple risk factors for HCC is additive, as the presence of co-infection with HBV/HCV and HBV/HDV increases the risk of HCC by a factor of 2–6. Alcohol abuse, on the other hand, increases this risk of HCC.

8.1.4.2 Pathogenesis of HCC

As explained in Sect. 8.1.4 above, risk factors for several etiologies are involved in the etiology of HCC. Chronic exposure to one or a combination of these risk factors causes fibrosis, cirrhosis, and eventually liver damage that can lead to HCC. It is commonly recognized that both intrinsic and extrinsic factors have a substantial impact on the progress of HCC. Hepatocytes undergo malignant transformation in the tumor's macroenvironment through mechanisms that limit tumor removal, avoid apoptosis, and promote tumor growth and angiogenesis. Cirrhosis causes carcinogenic changes and is observed in 90% of HCC patients. The carcinogenic non-cirrhotic process is responsible for the remaining 10% of individuals with malignancies. In patients suffering from chronic hepatic disease and cirrhosis, HCC is the utmost primary malignancies. The gut-liver axis (GLA) has recently received much attention for its role in the progress of HCC. The gastrointestinal system and liver have bidirectional anatomical and functional interactions that lead to this axis. In addition, a complex network of communications between the intestinal flora and the liver is important in the regulation of the HCC tumor microenvironment, revealing the liver to pathogen-associated molecular patterns such as bacterial lipopolysaccharide, DNA, peptidoglycan, and flagellin. Contributes to the etiology of HCC. In fact, changes in the gut flora break down the gut barrier, allowing Toll-like receptor ligands to reach the liver and activate the inflammatory response. In this it shows how new insights into the process of microbiota immunomodulation, denoted by synbiotics, affects HCC via GLA leads to new treatment options (Fig. 8.1).

8.2 The Role of Synbiotics in HCC

Controlling the host's human intestinal flora (GM), preventing intestinal toxinopathy-related endotoxemia, maintaining intestinal epithelial barrier function, suppressing the transfer of GM and pathogen-associated molecular patterns (PAMP) to the body circulation, etc. Multiple routes are all useful. To minimize the risk of developing HCC, synbiotics stimulate the development of useful bacteria that produce anti-inflammatory compounds that can reduce the oxidative stress of the liver of HCC by cumulative expression of antioxidant enzymes. In addition, by reducing obesity, synbiotics help prevent lipotoxicity in the liver. The

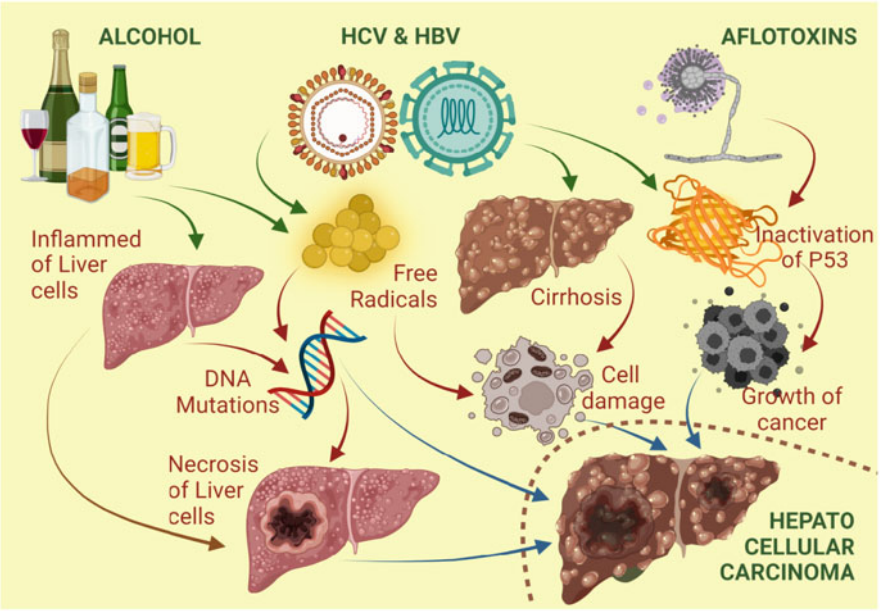

Fig. 8.1 Pathogenesis of HCC

anti-angiogenic activity of synbiotics is associated with downregulation of vascular endothelial growth factor (VEGF) and angiogenic factors VEGFA and angiopoietin (AP). Synbiotics have been claimed to be able to upregulate the manifestation of tumor suppressors while inhibiting the expression of oncogenes involved in HCC formation.

8.2.1 Synbiotics in Immune and Inflammatory Regulation (IIM)

IL-4, -5, -13 cytokines produced from Th2 CD4+ T cells, chemokines, and other chemical mediators regulates the hepatocellular immune response. A subsection of these T cells also produces IL-9. Subsequent cellular responses include recruitment and initiation of eosinophils, congenital group 2 lymphocytes (ILC2) and basophils, and changes in the epithelial barrier (Yu et al. 2016). The humoral immune reaction of kind 2 immunity is represented with the aid of using intently associated populations of CD4+ follicular T helper (Tfh) cells, IgE produced with the aid of using IL-four generating Tfh2 and IL-4 and -13 generating Tfh13 cells (Gowthaman et al. 2019). Building both of these adaptive immune responses begins with activating the appropriate type of antigen-presenting cells (APCs). This requires activation of the innate immune system, as APC should induce antigen-specific T cell resistance in the absence of activation signals. Tolerance is the primary response of the gut immune system to food antigens (Noah et al. 2019). Dendritic cells

(DC) recognize antigens (from hazardous microbes or food) that enter through cellular connections and trigger the adaptive immune system by governing T cell responses. In CCR7-dependent manner, DCs migrate to deliver either starting or bearing signals to naïve lymphocytes within gut-associated lymphoid tissues (GALT) (Worbs et al. 2017). Pathogen-associated molecular patterns (PAMPs), including as DNA, flagellin, and LPS, initiate nuclear factor kappa B and peptidoglycans via TLRs and nod-like receptors (Yiu et al. 2017). The probiotic bacteria's capacity to locally and broadly alter the host GIT mucosal immune system may be one of their most crucial properties. Certain species of gut commensal microbiota are necessary for the regulation of immune responses, and disruptions in the microbiota could result in a breakdown in immunological regulation, the proliferation of more pathogenic microorganisms, and the encouragement of inflammation. The generation of immunomodulatory responses by the interaction of probiotic microorganisms with the resident microbial flora, gastrointestinal epithelium, and intestinal immune cells is highly complex (Klaenhammer et al. 2012). Lipoteichoic acid (DMA), peptidoglycan, and S-layer proteins are found primarily in the probiotic microorganism MAMP (Bron et al. 2011).

Numerous studies have shown that anti-inflammatory IL-10 is increased and inflammatory IL-12 and TJMF-H are downregulated, while production of inflammatory cytokines is significantly reduced (Wang et al. 2012). The GI microflora promotes the function of the mucosal barrier and improves the host's immunity to intestinal infections. During active infection, IL-1β is a routinely produced cytokine and is important for neutrophil repair and pathogen eradication. Microflora plays an important role in the production of homeostatic levels of pro IL-1β in local intestinal macrophages (Kamada et al. 2013). *L. reuteri, L. acidophilus, L. casei, E.* Probiotics, including *Streptococcus thermophiles* and *bifidium*, motivate dendritic cells to produce COX-2, TGF-β, IL-10, and indoleamine-2,3-dioxygenase. It then increases the production of CD4 Foxp3 regulatory T cells (Tregs) and the suppressor activity of certainly occurring CD4CD25Tregs. They also reduce the responsiveness T and B lymphocytes and the number of T helper Th17, Th2, and Th 1, cytokines without prompting apoptosis, thereby inhibiting HCC (Kwon et al. 2010).

8.2.2 Impact of Synbiotics on IIM

In general, *Lactobacillus* (LAB), most frequently *Bifidobacterium* and *Lactobacillus* species, but also *Streptococcus, Lactococcus,* and *Enterococcus* species, as well as some yeast strains, are established probiotics that fit these criteria (Russo et al. 2022). In animal trials, a lot of different LABs have demonstrated the potential of probiotics. Several probiotics had been shown to be active in the treatment of IBD: *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Lactobacillus casei,* and *Lactobacillus acidophilus*. In recent years, there has been accumulating evidence that probiotic strains may exhibit the same activity as synbiotic bacteria, including immunomodulation (Masood et al. 2011; Tlaskalova-Hogenova et al. 2004; Borchers et al. 2009) (Fig. 8.2).

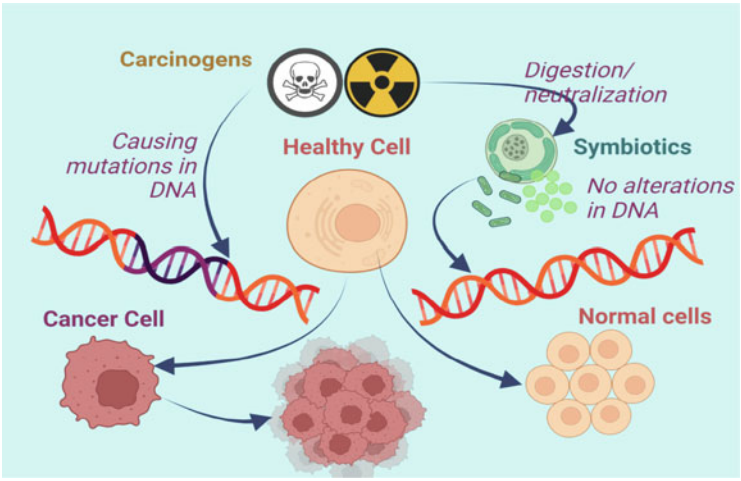

Fig. 8.2 Impact of synbiotics in cancer

8.2.3 The Role of Synbiotics in HCC

Oribacterium and *Fusobacterium* are the most usually recognized bacteria in the tongue swabs of HCC case patients. On the other pointer, the intestinal HCC microflora showed that the amount of *Bifidobacterium* spp., *Lactobacillus* spp., and *Enterococcus* spp. was reduced. In fact, recent studies had shown an association between specific bacterial profiles and HCC cases, showing more levels of *E. coli* and other Gram-negative bacteria in the gut flora associated with elevated serum LPS levels. In addition, a decrease in Verrucomicrobiota and an increase in actinomycetes were observed at the same time. In addition, in cases with NASH persuaded cirrhosis and HCC, increased concentrations of Bacteroides and Luminococcus, as well as increased concentrations of *Akkermansia* and *Bifidus*, compared to NASH caused cirrhosis without HCC. Detected patients with non-HBV and non-HCV (NBNC)-related HCCs have high levels of pro-inflammatory bacteria (*Escherichia coli, Enterococcus*) and low levels of pro-inflammatory bacteria (*Ruminoclostridium, Faecalibacterium, Ruminococcus*), resultant in anti-inflammatory bacteria. It also suggests that intestinal translocations may cause carcinogenesis. However, the functional special effects of the microbial flora on the development of HCC are associated with cirrhosis-related changes rather than specific HCC-related abnormalities that may exacerbate the progression of HCC (Fig. 8.3).

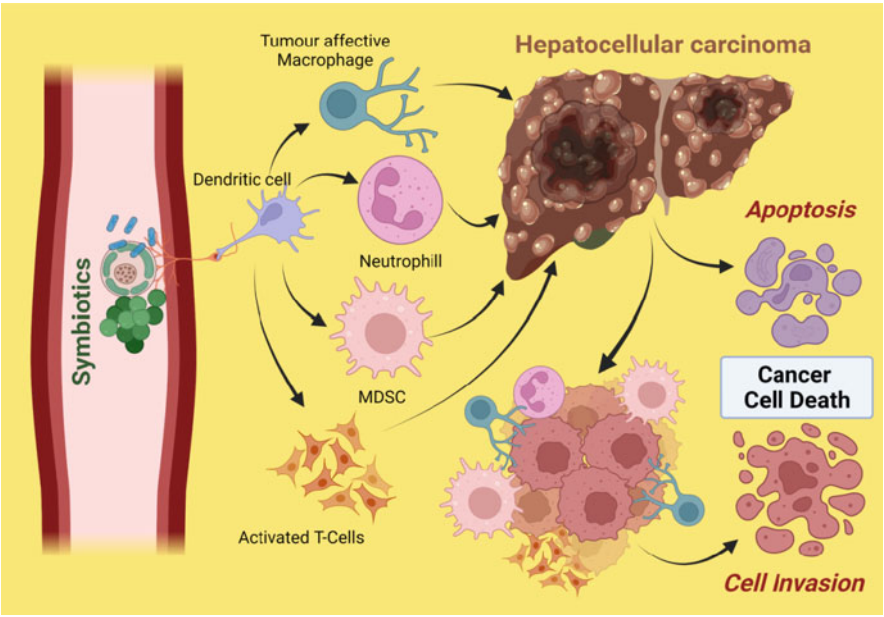

Fig. 8.3 Role of synbiotics on immune modulation in HCC

8.3 Synbiotics New Therapeutic Approach

The usage of prebiotics, probiotics, or both (synbiotics) is recommended for the treatment of NASH. Despite numerous publications in this area, determining the actual effect of probiotics on the prevention or treatment of NAFLD is difficult due to the use of various animal models and bacterial strains in experimental designs. Probiotics are highly regarded and improve liver function tests, but the 2007 Cochrane Collaboration Systematic Review didn't find an RCT. This suggests that it is difficult to upkeep or reject probiotics in the NAFLD due to lack of randomized clinical trials.

8.3.1 Previous Studies on Effects of Prebiotics and/or Probiotics on Liver Aminotransferase Ranges in NAFLD Case Patients

Aminotransferase was improved in studies with the highest dose and combination therapy in the treatment group. Improvements in aminotransferases cannot be clinically directly associated with improvements in NAFLD/NASH. However, this analysis focused on aminotransferases because the studies included lack of post-intervention liver biopsy and measurement of inflammatory markers. This was the only parameter tested in all three studies that showed changes after treatment. The

combination of treatment and lifestyle intervention significantly improved aminotransferases and reduced body mass index (BMI), but the prebiotic and probiotic doses and the combination of treatments were used in each study. No, it's difficult to compare. The combination of treatment and lifestyle intervention significantly improved aminotransferases and reduced body mass index (BMI), but the prebiotic and probiotic doses and the combination of treatments were used in each study. According to Aller et al. It's difficult to compare the number of cobination therapy and the intervention consisted of probiotics. The treatment group showed decreased AST and ALT levels, but no change in BMI. Marag Arnella et al. used interventions containing 10 times higher doses of probiotics, prebiotics, and lifestyle changes. The decrease in aminotransferase was significantly greater in both groups, as was the decrease in BMI. The improvement may be due to the effects of lifestyle changes rather than symbiotic therapy. Nevertheless, there are significant differences in AST levels between post-treatment groups, which may support the effectiveness of synbiotic therapy. In their study Wong et al. used a cheap probiotic dose in combination with a high prebiotic dose compared to Malaguarnera et al. Along with lifestyle interventions, no significant changes were observed in somewhat of the metrics despite the equivalent duration of treatment. It is noteworthy that the treatment duration of all studies was too short to show histological changes for therapeutic effect and reduced morbidity. However, Aller et al. had the shortest treatment period. This may suggest that probiotic administration has a dose-response effect and needs further investigation in future studies. Since only one of the studies included in this review contained such an analysis, an additional recommendation for future studies is to analyze pre- and post-treatment liver biopsies. Combinations of different probiotic species with a small number of samples were also included in the study and were difficult to compare. It will be interesting to capture changes in the gut microbiota after treatment, which is not documented in any of the studies reviewed in this study. Molecular tools are now available for analyzing the content of microbiota in health and illness.

In conclusion, microbiota showed to play an important part in the development of complications of liver disease, and there is evidence that it has a high fundamental involvement in the development of some liver diseases such as NASH and NAFLD. Increasing unfortunately, due to the lack of efficient clinical data, there are no recommendations for the use of probiotics in clinical practice for the time being. Longer term studies need to be directed to determine the safety of probiotics and synbiotics therapies in NAFLD. Probiotics and synbiotics have been shown to improve NALFD in a small number of human studies. However, the potential of probiotic treatment in NAFLD is investigated in larger, higher quality studies, as there is sufficient evidence to support the use of treatments, including regulation of the gut microbiota in the management of liver disease. Need to do it. In addition, metabolites produced by synbiotics through the breakdown of food and phytochemicals may help reduce the occurrence of HCC. The potential of synbiotics as an adjunct therapy for risk management and treatment of HCC has been demonstrated through these various anti-cancer pathways. As a final point, probiotic bacteria can bioconvert non-nutritive dietary ingredients like proanthocyanidins into

simpler compounds with anti-cancer properties at HCC. The development of synbiotics with enhanced anti-cancer capabilities may help develop diets and adjuncts as HCC prophylaxis strategies.

8.4 Conclusion

HCC is a serious public health issue and the fourth leading cause of cancer mortality worldwide. Multiple factors are involved in the progression of HCC. Balanced diet and optimal nutrition (increased consumption of fish, vegetables, white meat, coffee, (reduced consumption of fat, lean meat, alcohol), especially for people with liver disease (chronic disease). The relevance of (including) is noteworthy. Recently, specific geographical diet has been shown to minimize HCC risk, representing a new research paradigm. In addition, scientific research has shown the potential to produce symbiotic (probiotics and prebiotics) functional foods for cancer prevention. It goes without saying that more research work is obligatory to aptly identify the bioactive probiotic metabolites (postbiotics) of precise foods and phytochemicals as well as to comprehend the various mechanisms by which these postbiotics interact with the host and show that probiotics and prebiotics improve GM regulation and reduce liver carcinogens. In the future, the production of synbiotic or postbiotic combinations shows better potential anti-cancer effects that may help to develop nutritional strategies and adjuvant therapies to avert the progress of HCC.

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Synbiotics in Lung Cancer

9

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9.1 Introduction

Among the cancer types, lung cancer is considered to be the most common with a high mortality rate. The lung is an organ that is constantly connected with the external environment so there is a high risk of microbiome harboring and carcinogen attack. Active or second handed smoking is the major reason for lung cancer (Lukeman 2015). Lung cancer is generally categorized into two major types. One is less common and it is known as small cell lung cancer. In lung cancer, the tumors can grow and spread faster than the second type, hence it responds well to chemotherapy (Escalera et al. 2021). The second type is known as non-small cell lung cancer, it is the major type of lung carcinoma. There are three major subtypes of non-small cell lung cancer, they are adenocarcinoma where oncogenes are triggered in cells producing mucus. Squamous cell carcinoma occurs in the inner linings of the lung and large cell carcinoma where cancer can develop in any part of the lung

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(López et al. 2021). It is considered that the number of microbiomes present in our body is 10 times greater than the number of cells in our body. Of which, the colon contains 70% of the microbial flora. Some of the commonly found gut microbiota are *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* (Sagar et al. 2015). Imbalance in this gut microbial flora results in dysbiosis. This results in various diseases like bowel inflammation, lung cancer, hypercholesterolemia, etc. Scientists have figured out a plan to cure this disease using synbiotics. Synbiosis is the synergistic effect of both probiotics (live microorganisms) and prebiotics (substrate for the microorganisms) that positively affect the growth of gut microbial flora. When cancer patients undergo chemotherapy the inner mucosal layer of their intestine gets degraded. The microbial flora present in the gut produces a small chain fatty acid called butyrate which induces mucin secretion that helps to bring back the normal mucosal layer of the intestine (Sagar et al. 2015). It was reported that the saliva of the person who is affected with lung cancer contains the strains of *Neisseria*, *Streptococcus*, and *Porphyromonas*. This serves as the biomarker to detect lung cancer. Scientists believe that there is a strong connection between the gut and lung which is known as the gut-lung axis (Liu et al. 2021). The communication between the lung and gut is bidirectional. The metabolites produced by this microbial flora serve as an immune response to the host, thus maintaining systemic inflammation and immune homeostasis. Dysbiosis of *Firmicutes* and *Bacteroidetes* increases the risk of lung cancer. The presence of *Blautia obeum*, *La. Salivarius*, *Akkermansia muciniphila*, and *Coriobacteriaceae* indicate metastatic lung cancer (De Maria et al. 2021). Metastasis is a condition in which cancer cells migrate as packets from the primary tumor site to the secondary tumor site via blood or lymphatic vessels. Hence, synbiotics and fecal microbiota transplantation help to prevent and treat pulmonary disorders.

9.2 Lung Cancer

Lung cancer or lung cell carcinoma is a malignant tumor characterized by the uncontrolled growth of cells in lung tissues. It is the most common cancer that causes death in men and the second most common cancer in women, which is preceded by breast cancer (Mustafa et al. 2016). Lung cancer is the rarest disease during the First World War, and which popularized after the Second World War. The article which was published in the British Medical Journal in 1950, by Sir Richard Doll and Austin Hill, suspected that smoking can cause lung cancer and is an eye opener for all researchers. As suspected, people who smoke have the greatest risk of lung cancer, while people who never smoked are also susceptible to cancer. In 1985, the total death rate for lung cancer was 9,21,000 which is 17% more than the lung cancer death rate calculated in 1980 (Spiro and Silvestri 2005). By the data from Global Statistics 2020, lung cancer has occurred in 2.21 million people and resulted in 1.8 million deaths (Sung et al. 2021).

9.2.1 Causative Agents

The major reason for lung cancer is smoking. A lifetime smoker has a 20–30 times increased risk of lung cancer than non-smokers contributing to nearly 85% of the lung cancers (Minna et al. 2002). Cigarettes contain 73 known carcinogens which include polycyclic aromatic compounds like benzo-alpha-pyrene, NNK [Nitrosamine 4(methylnitrosamino) 1-(3pyridyl)-1-butanone], a radioactive isotope of Polonium 210, etc. (Mustafa et al. 2016). Micro dissection of epithelial tissues of a former smoker and current smoker with lung cancer showed thousands of lesions than the lung of a non-smoker (Spiro and Silvestri 2005). So far smoking cannabis is not proven as a risk factor for lung cancer (Mustafa et al. 2016). The remaining 10–15% of lung cancer patients are non-smokers. They are either inherited genetically or when they are exposed to carcinogens including asbestos, nitrogen dioxides, sulphate aerosols, indoor and outdoor air pollution containing 2.5 PM (Particulate Matter). Polymorphism of chromosomes 5,6 and 15 can increase the risk of lung cancer. Miscellaneous substances like metals (aluminum, cadmium, chromium (VI), beryllium, etc.), ionizing radiation (X-rays, gamma rays, plutonium), soot resulting due to incomplete combustion, MOPP from paint and toxic gases [methyl ether, bis (chloromethyl) ether, etc.] can also cause lung cancer (Mustafa et al., 2016).

9.2.2 Mode of Action

When the carcinogens enter our body, they bind directly to the DNA. These adducts cause mutation like guanosine to thymine transversion which may turn a normal cell into a cancer cell (Spiro and Silvestri 2005). The pathogenicity of lung cancer is mediated by the activation of proto-oncogenes and the inhibition of tumor suppressor genes. C-myc, cyclin 1, BCL2 gene mutations can trigger proto-oncogenes. Mutation in signaling pathways, especially K ras mutation has a 10–30% chance of converting proto-oncogene into an oncogene. The tumor suppressor gene gets inactivated by methylation, histone tail modification, and micro-RNA regulation. Overexpression of Endothelial Growth Factor Receptor (EGFR) plays a major role in cancer development. It induces cell proliferation, angiogenesis, tumor invasion (metastasis), and resistance to apoptosis. Mutations in genes like C-MET, NKX2–1, LKBI, PIK3CA, and BRAF exhibit similar kinds of activity (Mustafa et al. 2016). The presence of telomerase RNA and catalytic component (hTERT) in cancer cells makes them immortal (Minna et al., 2002).

9.2.3 Symptoms

People with lung cancer have the following clinical manifestations. Respiratory and systemic symptoms like cough, shortness of breath, and chest pain that occur due to cancer mass in the lungs, pressing adjacent structures. Ectopic ADH (antidiuretic hormone) and ACTH (adrenocorticotrophic hormone), and other symptoms like

hypercalcemia, invading of Pancoast tumor (tumor on top of the lung) in the sympathetic nervous system, Horner syndrome (dropping of the eyelid and small pupil) can be observed in lung cancer patients (Mustafa et al. 2016). One of the major drawbacks of lung cancer treatment is late detection since most of these symptoms are shown during III B or IV stages (Spiro and Silvestri 2005).

9.2.4 Diagnosis

People with such symptoms are diagnosed through sputum cytology, and they are histologically diagnosed by the following technologies. One of the earliest methods of diagnosis was chest radiography which was performed from 1970 to 1980. This method failed to show a reduction in lung cancer after chemotherapy, and it is rejected as it causes contamination. Followed by which people started using and still rely on computed tomography (CT scan) that is sensitive to visualize pulmonary nodules and non-calcified nodes in the lungs. But there are some chances of getting false-positive results which may be due to increased time length and overdiagnosis. The non-surgical biopsy technology is Positron Emission Tomography (PET); it is also one of the diagnostic tools that are based on the biological activity of a neoplastic cell. Tumor cells uptake more glucose and perform extensive glycolysis than normal cells, so PET uses radiolabeled glucose analog 2-[F-18]-fluoro-2-deoxy-D-glucose (FDG) to detect cancer cells for both intra and extra-thoracic analysis (Spiro and Silvestri 2005).

9.2.5 Types of Lungs Cancer

Based on treatment methods, lung cancer is majorly divided into two types. They are non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). The NSCLC is the majority type of lung cancer (80–85%). It is subdivided into three sub-classes. They are adenocarcinoma that is developed in the periphery of the lung (Lukeman 2015). These adenocarcinomas arise from the progenitor cells of bronchioles (Clara cells), alveoli (type II pneumocytes), and mucin-producing cells. Next is squamous cell carcinoma which arises from the central part of the lung, that is from the segments of the bronchi, and it is extended to the main stem bronchus and the lobes of the lung. In well-developed stages, intracellular bridging, squamous pearl formation, and individual cell keratinization are clearly seen. Large cell lung cancer is the rarest form of NSCLC which is found in the periphery although it is centrally located. It is a large necrotic tumor that contains sheets and nests of polygonal cells with vesicular nuclei and prominent nucleoli. The next type of lung cancer is small cell lung cancer where 30,000 new cases are reported every year. It is usually located in the peri-bronchial region. This SCLC compresses the circumference of the inner lining of the bronchus, and it is generally metastasized through the external lymph node. These tumors are white-tan, soft, and friable. It

also has three subtypes. They are oat cell lung carcinoma, intermediate cell type, and combined oat cell lung carcinoma (Travis 2011).

9.2.6 Treatment Strategies

Depending on the intensity of cancer and the performance status of cancer patients, lung cancer can be categorized into different stages. The treatment of lung cancer includes palliative care, surgery, chemotherapy, and radiotherapy. The first surgery for lung cancer was made in 1821 by an American "Milton Anthony" who surgically removed ½ pound of lung tissue along with 2 ribs just for 1-year survival. Lung cancer patients undergo different types of surgery including wedge resection having a high chance of recurrence, and contralateral resection which has a 55% of survival chance for 5 years (Spiro and Silvestri 2005). Our right lung has 3 lobes and the left has 2 lobes (Lukeman 2015). When patients are diagnosed with a small tumor on these lobes, it is removed through lobar excision or lobectomy. Usually, it is omitted because of concomitant disease risks (Spiro and Silvestri 2005). Radiotherapy is preferred to control tumor growth and reduce the chance of recurrence. Radiotherapy is compromised between adequate doses to minimize lung toxicity. The British Medical Research Council has devised Continuous High fractioned Accelerated Radio Therapy (CHART), where 1.5 Gy (Gray) is administered at eight-hour intervals to a total of 54 Gy, along with conventional daily treatment had striking effects in squamous lung carcinoma patients. 60 Gy is the standard radical dose for NSCLC patients. Radiotherapy has an 80% success rate for palliative lung cancer symptoms that include bone pain, hemoptysis, cough, and superior vena cava syndrome (Spiro and Silvestri 2005). Chemotherapy is given based on the patient's age, response, and willingness to take chemotherapy. Modern chemotherapy with carbo or cisplatin along with vinorelbine, gemcitabine, and taxane has shown easy administration with lesser side effects like nausea, vomiting, hair loss, and increased survival rates. Mutation in EGFR could be prevented by two anti-tumor oral inhibitors: gefitinib and erlotinib which are small anti-tumor agents that selectively inhibit the intracellular tyrosine kinase activity of EGFR. The antiangiogenic drug known as thalidomide is used to stop lung cancer progression (Spiro and Silvestri 2005). Sometimes a combination of one or more treatments is suggested based on the condition of the patient. For instance, to treat NSCLC either surgery resection in mediastinum involving lymph node is associated with neoadjuvant chemotherapy or chemotherapy is combined with radiography. A combination of one or more chemotherapeutic drugs is also suggested, for example, a patient with an extensive stage of SCLC is treated with the combination of drugs such as cisplatin, etoposide, irinotecan, topotecan, or paclitaxel whose action shrink the tumor, reduce symptoms, and extended rate of survival from 10 to 16 months (Minna et al. 2002).

9.3 Synbiosis

9.3.1 Gut Microbiome

The gastrointestinal tract of our body contains 10^{14} bacterial cells which are 10 times greater than the number of cells in our body. Hence, it is known as the second genome of humans. Usually, a newly born baby has a sterile gut, but when it is exposed to the external environment, mother's milk, and solid foods their gut is harbored with microbes. Even though our body contains many microbes, nearly 70% of them are harbored in the gastrointestinal tract because of the availability of nutrients. These microbes that coexist with the human body (host) are termed gut microbial flora. This microbial flora confers many health benefits to the host like increased oral health, decreased respiratory infections, increased healing process in acute distal radial fracture, and an increase in the mental health of dementia and meningitis patients (Ale and Binetti 2021). Some common examples of these gut microbiota are *Lactobacillus* and *Bifidobacterium* (LAB); sometimes *Firmicutes* are added to this list. The imbalance in this microbial content (dysbiosis) can cause diseases like inflammatory bowel syndrome, antibody-associated diarrhea, colon cancer, hypercholesterolemia, etc. (Vyas and Ranganathan 2012). Dysbiosis of microbial flora also leads to metabolic disorders. For example, a decrease in *Bacteroides* viable count in the gut reduces the digestion of polysaccharides entering our gut. Likewise, decrease in fibro lytic microbes like *Eubacteria*, *Bifidobacterium*, and *Faecalibacteria* reduce starch and sucrose metabolism, pyruvate and galactose metabolism, and metabolic processes like glycolysis and gluconeogenesis (Ale and Binetti 2021). So, it is important to maintain these microbes in our gut. This can be achieved through supplements like probiotics and prebiotics.

9.3.2 Probiotics

According to WHO, probiotics are non-pathogenic live microbe(s) that when administrated in adequate amounts confer health benefits to the host (Vyas and Ranganathan 2012). When Vergin and co-workers were studying about the detrimental effects of antibiotics, he coined the term probiotics from *probiotika* (a substance that survived antibiotics) (Pandey et al. 2015). The microbes that are viable, stable, storable, and which are GRAS (Generally Regarded as Safe) certified are selected as probiotics. Some examples of these health conferring microbes are *Bifidobacterium*, *Lactobacillus sp*. like *Lactobacillus rhamnosus*, *L. reuteri*, *L. casei*, *L. acidophilus*, *Bacillus coagulans*, *Enterococcus faecium,* and *Saccharomyces boulardii* (Pandey et al. 2015).

9.3.3 Prebiotics

Prebiotics are non-digestible fibers (an oligomer made up of 4–6 monomeric hexose units) that are fermented by microorganisms in the gut as the source of nutrition (Vyas and Ranganathan 2012). Some other prebiotics includes breast milk, soybean, raw oat, unrefined wheat or barley, chicory like inulin, and non-digestible carbohydrates like trans-galactooligosaccharide (GOS), fructooligosaccharide (FOS), and xylooligosaccharide (XOS). Prebiotics can prevent diarrhea, constipation, flatulence, etc. Prebiotics should be resistant to bile salts, gastric acid, and hydrolyzing enzymes. The microbiome in the gut should be able to ferment them easily. Generally, probiotics are active in the small intestine and prebiotics are active in the large intestine (Cerezuela et al. 2011).

9.3.4 Benefits of Gut Microbiome

Some highlighting functions of gut microbial flora, in the presence of probiotics and prebiotics, are as follows. Gut microbial flora can synthesize Small Chain Fatty Acid (SCFA) like acetate, propionate, butyrate, etc. which is involved in mucin production, growth, and differentiation of other microbes, regulation of hepatic lipogenic enzyme, accessing genes to transcriptional factors by acetylating histone tail and reversal of cells from neoplastic to non-neoplastic phenotype (Vyas and Ranganathan 2012). It can synthesize derivatives of vitamin B and amino acids, and it is involved in the biotransformation of bile salts which is important for glucose and cholesterol metabolism (Sagar et al. 2015). This microbial flora competes with pathogens for nutrients and attachment to alimentary canals either by producing antimicrobial compounds against them or by producing inhibitory compounds like hydrogen peroxide which may block or degrade the toxin receptors on the pathogen. It increases anti-inflammatory responses like enhanced IgA secretion and increased lymphocyte and leucocyte secretion in GALT and periphery blood vessels to destroy the invaded pathogen (Pandey et al. 2015). This function varies from person to person since the microbial composition varies (Vyas and Ranganathan 2012). It differs based on various factors like age, sex, diet, lifestyle, location, and functions of the immune system (Ale and Binetti 2021). Some of these functions are commonly observed in synergetic conditions prevalent during the therapeutic management using probiotics and prebiotics in a synbiotic manner.

9.3.5 Synbiotics

Synbiosis (*syn* means together and *biotic* means for life) is defined as the synergistic beneficial effect of both probiotics and prebiotics. This idea was proposed by Gibson to improve the implantation and survival rate of live microorganisms in the gastrointestinal tract. When two supplements (probiotics and prebiotics) are combined, their synergistic effect is greater than the sum of individually administered

supplements (Cerezuela et al. 2011). Synbiosis is introduced to overcome the survival difficulties faced by prebiotics. Administration of synbiotics increases the concentration of *Lactobacillus* and *Bifidobacterium*, and it helps to maintain the gut microbial balance. It improves the liver function of the liver cirrhotic patient. It increases immuno-modulating ability, prevents bacterial translocation, and reduces nosocomial infection caused during hospitalization or surgery (Pandey et al. 2015). The genome sequence and annotation of probiotics used in synbiosis should be publicly available for safety, identity, and purity. The synbiotics should commonly satisfy both autochthonous (resident/colonizing inside host) and allochthonous (microbes from an external source such as probiotics) microbes (Swanson et al. 2020). Synbiosis can be divided into two types, namely complementary synbiotics and synergistic synbiotics. In complementary synbiotics, the effects of probiotics and prebiotics are independent of each other. For example, the microorganisms which are used as probiotics adhere to the intestinal walls to confer health benefits, and the prebiotic aids in the growth of autochthonous microorganisms (Ale and Binetti 2021). Hence, they work separately. For example, the probiotic strain of *Lactobacillus acidophilus* increases the abundance of beneficial microbial flora such as *Akkermansia spp.* and *Lactobacillus spp*. in our gut. But in the case of synergistic synbiotics, the prebiotics is designed in such a way that they can provide nutrients to the co-administered probiotics. So, their effect of conferring health benefits is increased (Swanson et al. 2020). Some of the well-known functions of synbiotics are listed below. *B.coagulans* + inulin, when administered for 6 weeks reduces the c-reactive protein (a marker for inflammatory signs in our body) and increases glutathione (an antioxidant) levels. (*Lactobacillus* and *Bifidobacterium* + 10% FOS) can suppress systemic and intestinal inflammations. (*L. acidophilus* + rice bran) regulate hypercholesterolemia. Synbiotics prevent azoxymethane-mediated suppression of NK cell activity in Peyer's patches. Synbiotics are widely used to suppress cancer cells or to inhibit the recurrence of various types of cancers. *B. longum* + FOS and inulin inhibit pre-neoplastic lesion formation, and it suppresses mammary and colon cancer (Pandey et al. 2015). Probiotic *L. casei* is used to prevent the recurrence of bladder cancer. Prebiotics like inulin/FOS are used to reduce precancerous lesions and increase defense functions like secretion of IL-10, NK cells, etc. (Vyas and Ranganathan 2012).

9.4 Synbiotics in Lung Cancer

9.4.1 Gut Lung Axis

The complex interconnection between our gut and lung is known as the "gut-lung" axis. The cross talk between gut and lung occurs via microbiome and immune response. Though the communication between them is bidirectional, the cross talk between the gut to the lung is most common. (Liu et al. 2021) The gut harbors different bacteria like *Firmicobacteria*, *Bacteroidetes*, *Eubacteria*, *Ruminococcus*, *Faecalibacterium*, etc. When a pathogen enters the small intestines, the cell wall

fragments or protein parts of the microbes escape cytokines/chemokines and travel to cisterna chyli via the mesenteric lymphatic system and from there it reaches the lungs by entering the circulatory system (Liu et al. 2019). When it reaches the lung region, an immune response like dendritic cells, macrophages, and T cells are produced and get differentiated. This pathway can be altered in a different way also, that is, in the mesenteric lymphatic system antigen-presenting cells recruits naive B cells and T cells. These B cells get matured (plasma B cells) and synthesize immunoglobulins which leave the lymph node and enter into mucosal tissues to spread immune information. This process switches off the innate ability to produce IL-10 and anti-inflammatory molecules. This initiates dendritic cells to migrate to local lymph nodes to differentiate T cells. Later these differentiated T cells migrate to GALT (Gut Associated Lymphoid Tissue), which distributes them to the mucosal (bronchus) and periphery non-mucosal layer, thus improving immune response against the pulmonary pathogen. Similarly, an immune response from the lungs can travel to the gut as well. The lungs, which are considered sterile organs, harbor air-borne microbes below the vocal cord and in bronchoalveolar lavage fluid. The upper respiratory tract (nostrils) contains *Firmicutes* and *Actinobacteria*. The lower respiratory tract (lungs) harbors *Proteobacteria*, *Bacteriodetes*, and *Firmicobacteria*. During the cross talk between lung and gut, the dendritic cells present in the lungs imprint the expression of gut homing integrin (α4β7 and CCR9) on T cells, which initiate T cells migrate to the gut to elicit an immune response (Bingula et al. 2017).

9.4.2 Dysbiosis of Microbiome in Lung Cancer Patients

Since gut and lungs are interconnected, the dysbiosis of gut microbial flora may increase the chance of lung disease. The dysbiosis of the gut microbiome leads to the progression of lung cancer through genotoxicity, systemic inflammation, and defective immune surveillance. Imbalance in *Firmicutes* and *Bacteroides* increases the risk of lung cancer. In general, the gut microbiome of lung cancer patients shows increased *Bacteroides*, *Enterococcus*, *Fusobacterium*, *Prevotella*, *Ruminococcus*, *Veillonella*, and decreased *Bifidobacteria*, *Blautia*, *Coprococcus*, *Dialister*, *Escherichia*, *Enterobacter*, *Faecalibacterium*, *Firmicutes*, *Lachnospiraceae*, *Shigella*, and phylum of *Actinobacteria*. Microbes like *Blautia obeum*, *Lactobacillus salivarius*, *Akkermansia muciniphila*, and *Coriobacteriaceae* are overgrown in lung cancer subtypes and metastatic patients (Liu et al. 2021). An experimental study, which was conducted on 16 healthy individuals and 30 lung cancer patients based on the three tumor biomarkers (CYFRA 21-1, NSE, and CEA) showed the following results. There was a decrease in *Firmicutes*, *Bacteroides*, and *Actinobacteria* in adenocarcinoma patients. Similarly, squamous epithelial lung cancer patients showed decreased *Prevotella spp*. The NSCLC patients showed increased *Proteobacteria* and *Verrucomicrobia* and recurrent adenocarcinoma patients show increased *Fusobacteria* and *Streptococcus*. Certain microbes are commonly found in all groups of lung cancer they include *Bifidobacterium*, *Blautia*, *Dialister*,

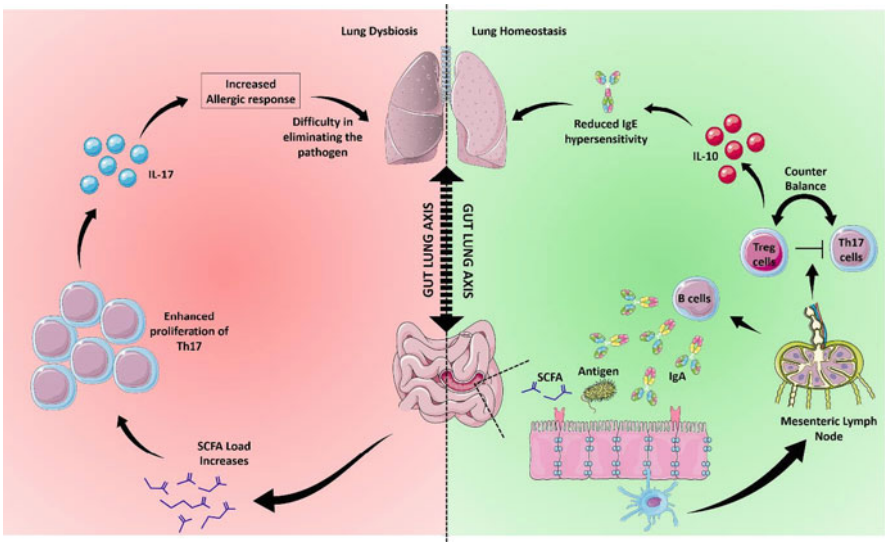

Fig. 9.1 Illustration of lung dysbiosis and homeostasis: In the lung dysbiosis, SCFA (Small Chain Fatty Acids) takes the lead in mediating the pro-inflammatory responses resulting in loss of immune-homeostasis. In a healthy state, the immune cells take over the production of anti-inflammatory markers ensuring a steady state of immune-homeostasis

Lachnospiraceae, and *Ruminococcaceae* (Liu et al. 2019). Hence, the composition of certain gut microbial flora serves as a biomarker for the diagnosis of different types of lung cancer. It helps to identify different stages of tumor, and it also helps to monitor the recurrence, prognosis, and metastatic condition of lung cancer patients after treatment. It is suspected that the tumor cells might synthesize certain metabolic products or create differential profiles that may favor selective adherence of some members of microflora to the gut (Liu et al. 2019) (Fig. 9.1).

9.4.3 Probiotic Effect on Lung Cancer

Restoration of these depleted microbes back into the gut can either resist or reduce the effects caused by lung diseases (Liu et al. 2021). This can be achieved through probiotics, whose administration has positive effects on lung cancer patients. The evidence of probiotic influence on lung cancer was found in 1985, and it has recently emerged due to increased results. When mice C57BL16 is injected with *Bifidobacterium infantis*, it increases the necrosis rate of lung cancer cells, thus prolonging the survival period (Zhou et al. 2022). When the Lewis lung cancer model is treated with cisplatin and ABX (an abiotic cocktail of vancomycin, ampicillin, and neomycin), it increased the tumor size and decreased the survival rate. Here the ABX upregulates VEGF (Vascular Endothelial Growth Factor) that induces angiogenesis in tumor cells and downregulates apoptotic promoters BAX

and CDKNIB. But when it is treated along with probiotic *Lactobacillus acidophilus*, it resulted in reduced tumor size due to upregulation of IFN gamma and granzyme B, thus increasing the survival rate (Gui et al. 2015). Similarly, when the mouse melanoma model is treated with a *Bifidobacterium* cocktail (*B. bifidum*, *B. longum*, *B. lactis*, *B. breve*), it showed control against tumor which is as same as PD-L1 (Programmed Death Ligand 1)-specific antibody therapy. A probiotic strain of *Parabacteroides* and *Methanobrevibacter* results in the downregulation of cancer cell proliferation and thus increases the survival rate of lung cancer patients.

9.4.4 Synbiotic Approach to Reduce the Effect of Chemotherapy

Increased use of antibiotics like penicillin, cephalosporin, and quinolones can increase the risk of lung cancer. When lung cancer patients undergo antibiotic-associated chemotherapy, their gut microbial flora gets degraded along with their intestinal walls, which results in severe diarrhea. When these patients are supplemented with a probiotic strain of *Clostridium butyricum*, the patient showed a reduced effect of diarrhea and other inflammatory bowel diseases. In general, the depletion of Firmicutes and Bacteroides colonies in the gut reduces the production of SCFA (Liu et al. 2021). SCFA are Small Chain Fatty Acids, synthesized either by autochthonous or allochthonous microbes by degrading natural dietary fibers present in the colon and caecum or by degrading prebiotic fibers like inulin, FOS, etc. These SCFAs are essential for our body because it nurtures intestinal epithelial cells and exhibits an anti-inflammatory effect (Zhou et al. 2022). Some common examples of this SCFA include acetate, propionate, and butyrate. The NSCLC patients show dysbiosis of butyrate-producing microorganisms like *Faecalibacterium prausnitzii*, *Clostridium leptem*, *Clostridial cluster I*, *Clostridial cluster XIVA*, *Rumminococcus sp.*, and *Roseburia sp*. (Gui et al. 2020). These butyrate- producing microorganisms synthesize a compound called mucin which helps in the regeneration of the ruptured mucosal layer of the intestine. Variations in this SCFA like sodium butyrate induce apoptosis in tumor cells or act as histone deacetylase inhibitors (Gui et al. 2020). Hence, synbiotic administration of these depleted microbes along with a suitable substrate can reduce the risk of lung cancer. For example, *Bifidobacterium* (probiotic) supplied with inulin or oligo fructose (prebiotic) restores *Bifidobacterium* (Liu et al. 2020). Sometimes a mismatched selection of probiotics or prebiotics for the growth of microbes can cause adverse effects. For example, synbiotics with *Bacillus* strain is harmful because of their ability to generate multiple toxins as it harbors mobile antimicrobial resistance gene. So, care should be taken while designing synbiotics for lung cancer patients. However, the interaction between gut and lung is undefined. Acute exposure of a single dose of intra-tracheal LPS in the airways of mice has been transported into the bloodstream within 24 h. Similarly, when mice are subjected to inhalation of a non-absorbable tracer, the gastrointestinal tract of mice showed a trace amount of this non-absorbable element within a few hours of inhalation (Bingula et al. 2017). Hence, there is always a debate whether lung cancer

is the product of gut microbial variation or vice versa. Scientists are on their way to discover genetically modified microorganisms to enhance the efficacy of anti-cancer treatments. It can also be used as a delivery vehicle to administer chemotherapeutic agents (Scott et al. 2018).

9.5 Conclusion

Synbiotics have a promising potential for the prevention and treatment of cancer. Yet the research studies are still not sufficient to substantiate the significance of synbiotics. There is evidence to showcase the beneficial effects of probiotics and prebiotics. But it is still difficult to conclude the role of these agents in the treatment of cancer. On the other hand, the use of prebiotics and probiotics as supplementary therapy in cancer treatment has been demonstrated in several reports. Research on lung microbe population in in vivo experiments dealing with the complex interaction between the microbes and the host may provide insights into future studies. Synbiotics and the metabolic products of probiotics can be produced economically and has fewer side effects, as there is no evidence or reports stating that ingested probiotics can pose risk to the patients. Probiotics in the balanced diet were also found to be beneficial in the prevention of cancer, but worldwide people prefer foods containing high fat and low fiber, which is always a threat to cancer-associated risk factors including obesity and diabetes. However, the effect of pro- and prebiotics to combat lung cancers concerning the gut-lung axis is more likely to depend on the host microbiota, duration of pro- and prebiotic consumption (Minimum of 6 days and maximum of 1 year), and the age of the patients. Further clinical studies investigating the role of synbiotics and their impact on lung cancer will pave way for the therapeutic management of cancer.

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Human Microbiome Modulation: A Potential Therapeutic Strategy for Pancreatic Cancer

10

Arghya Kusum Dhar

10.1 Introduction

One of the most fatal malignancies is pancreatic cancer. It is currently the world's fourth largest cause of cancer-related death, but that number is expected to rise to the world's second leading cause by 2030 (Rahib et al. 2014). Pancreatic ductal adeno carcinoma (PDAC) and pancreatic neuroendocrine tumour (PNET) are the two main forms of tumours in pancreatic cancer. A PDAC tumour is an exocrine tumour that originates in the pancreatic ducts, whereas a PNET is an endocrine tumour originates from the pancreatic islets that can secrete numerous hormones, such as insulin and glucagon (Stark and Eibl 2015; Alkassis et al. 2021; Ro et al. 2013). The overall survival rate of PDAC is about 9% (Siegel et al. 2018), despite the fact that therapy advances are ongoing. Only a small percentage of people with pancreatic cancer are surgical candidates. The 5-year survival rate in such situations is about 25% (Geer and Brennan 1993). The absence of initial clinical symptoms, metastatic spread, chemotherapy resistance, and a high relapse frequency following surgery are all factors that contributed to this dismal result. In addition, PDAC has a unique tumour microenvironment that is composed primarily of fibroblasts and protumoral immune cells (Vitiello et al. 2019).

Pancreatic cancer has some known causes, such as high-fat diet, obesity, type 2 diabetes, pancreatitis, and smoking; however, the primary genetic threat factors are still mostly unidentified at this point. Only a small proportion of PDAC patients have mutations in the Breast Cancer Gene 2 (BRCA2) protein and other DNA damage repair proteins (Alkassis et al. 2021). Some 10–20% of patients with PNET type tumours have familial diseases like multiple endocrine neoplasia syndrome

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1 (MEN1), von Hippel-Lindau disease (VHL), neurofibromatosis type 1 (NF-1), and tuberous sclerosis (TSC) (Ro et al. 2013).

Although current advances in surgery, chemotherapy, and radiotherapy treatments; pancreatic cancer continues to be a disease with a deficient prognosis, yet when originally resectable. There is a lack of biomarkers that can identify this cancer in its initial or preinvasive stages, as well as inadequate treatments, relapses, and delayed diagnoses are all contributing factors to this situation. Furthermore, pancreatic inflammation is regarded a significant risk factor, and chronic pancreatitis can up to 20-fold increase the risk of PDAC (Lowenfels et al. 1993). Additionally, the poor prognosis can be attributed to the complicated biology surrounding the microenvironment of a large desmoplastic PDAC tumour, which results in hypovascularity, hypoxia, inadequate drug delivery, and unsuccessful therapy (De Sousa Cavalcante and Monteiro 2014; Thomas and Radhakrishnan 2019). Moreover, despite major improvements in immunotherapy for the management of a wide range of cancers, PDAC immunotherapy development has proved difficult.

Most pancreatic cancers go undetected until they are advanced stages because of unclear symptoms like back discomfort and loss of appetite, which are commonly misdiagnosed as being caused by other conditions. Lack of understanding of the disease's pathophysiology, ineffective techniques for early detection and prevention, aggressive tumour biology and early metastases, as well as chemotherapy resistance have contributed to this disease's high mortality rate. Thus, there is a clear necessity for novel screening, preventive, and treatment methods for pancreatic cancer. As many as 10–20% of human malignancies are linked to the presence of microbes, which are capable of promoting carcinogenesis and are fundamental part of microbiota (De Martel et al. 2012). The estimated number of microbiota which is a collection of bacteria, archaea, viruses, fungus, and protozoa dwelling in the human body ranges from 10 to 100 trillion (Costello et al. 2009). The collective genetic material contained by these bacteria, recognized as the microbiome, greatly surpasses the human genome (Turnbaugh et al. 2007). Nonetheless, the greater parts of scientists use "microbiota" and "microbiome" interchangeably. Microbiota can be found on the skin, mouth cavity, conjunctiva, and genitourinary tracts in addition to the gastrointestinal tract, where the most of microorganisms inhabit (Sender et al. 2016).

The majority of them is safe and participates in numerous physiological processes, including nutritional absorption, vitamin and energy substrate production, immune system modulation, and/or protection against pathogenic microorganisms (Akshintala et al. 2019). Nevertheless, abnormalities in microbiota composition, frequently recognized as dysbiosis, are linked to a variety of disorders, including diabetes, obesity, and inflammatory bowel disease (IBD). In addition, the role of dysbiosis in malignancies such as laryngeal, gastric, colorectal, and liver cancer has been highlighted in recent years (Lozupone et al. 2012; Daniluk et al. 2017). Microorganisms such as human papillomaviruses, hepatitis viruses, and *Helicobacter pylori* have been associated to human malignancies (De Martel et al. 2012). Additionally, dysbiosis can have both favourable and negative effects on tumour response to therapy (McAllister et al. 2019). The microbiota can stimulate

inflammatory responses, affect the tumour immunological milieu, modify tumour metabolism, and modulate tumour sensitivity to drugs (Maekawa et al. 2018; Yu et al. 2020; Viaud et al. 2013b; Ma et al. 2018; Geller et al. 2017). Emerging evidence suggests that dysbiosis is also intimately associated with pancreatic oncogenesis.

The term "oncobiome",created to represent the area of study looking into the function of the microbiome in human cancer progression, has grown rapidly in the last few years (Yu et al. 2021). Initially focusing on colorectal cancer, the subject of oncobiome research has swiftly spread into other malignancies particularly pancreatic cancer. Some oncobiome-related cancer development mechanisms have been hypothesized. For example, bacterial toxins and/or metabolites have been shown to directly affect cancer genesis and progression (Yu et al. 2021), as well as modulating the host's local and systemic immune response (Yu et al. 2021). Also, microbial metabolism has been shown to be affected by oncobiome interactions (Yu et al. 2021). It is possible that these host–microbe interactions in cancer formation could take place both locally and remotely (Yu et al. 2021).

It is increasingly becoming clear that harnessing the influence of microbiota on the host could be a useful method for improving cancer treatment (Yu et al. 2021). It is imperative that researchers continue to investigate causes of cancer development and poor response to existing treatment of pancreatic cancer, as it remains one of the most lethal tumours in the world, and studies of the pancreatic oncobiome are still in their infancy.

This chapter emphasis on the influence of human microbiome on pancreatic cancer. Besides impact of gut microbiome changes, influences of pancreatic, skin, oral and lung microbiome on pancreatic cancer are also discussed. Interdisciplinary strategies for modifying the gut microbiota in pancreatic cancer with prebiotics, probiotics, postbiotics, synbiotics, next-generation probiotics, and faecal microbiota transfer (FMT) are also reviewed.

10.2 Epidemiology and Risk Factors of Pancreatic Cancer

Data on cancer deaths in 23 world regions was compiled in 1999 by Parkin D et al. by comparing cancer registries. Pancreatic cancer was found to be the 9th men and women's major origins of cancer-associated mortality and in terms of mortality; it is the 13th major reason of cancer-associated death. Due to the dismal prognosis, the death to incidence ratio was 98% in industrialized countries and 96% in underdeveloped ones (Capasso et al. 2018).

According to GLOBOCAN 2018 estimates, among malignancies, pancreatic cancer is 14th most prevalent worldwide, based on the number of new cases each year. When it comes to incidence rates, there isn't much difference between men and women. A dismal prognosis and nearly as many deaths (432,000) as cases (459,000) make pancreatic cancer 7th leading reason of cancer death in both males and females (Capasso et al. 2018).

Cancer Statistics Review (CSR) reports that pancreatic cancer incidence is rising (Capasso et al. 2018). Several experts estimate that incidence and death would continue to rise in Taiwan (Tseng et al. 2017).

By 2030, in Germany, pancreatic cancer will become the second leading cause of cancer-related fatalities, after breast and colorectal cancer, according to Quante AS et al. (Quante et al. 2016); the same pattern may be observed in the United States, where it is anticipated that pancreatic cancer would become the second biggest cause of cancer-related death, after lung cancer (Rahib et al. 2014). New cases and deaths in Italy reached over 13,000 in 2017; the incidence was 22/100,000 new cases per year in 2016 and is increasing both sexes, but more so in males (AIOM et al. 2018).

Eighty percent of those diagnosed with pancreatic cancer are above the age of 60, with the typical age being 71 years when diagnosed (Gold and Goldin 1998). Pancreatic cancer is more common in people who smoke, are obese, or have diabetes, according to a population-based epidemiological study (Gordon-Dseagu et al. 2017). Unlike other gastrointestinal tumours, pancreatic cancer risk factors are poorly understood and only explain 40% of cases. 10% genetic, 90% environmental (modifiable) factors (Becker et al. 2014).

Hereditary/genetic risk factors are

(a) *Hereditary Breast and Ovarian Cancer Syndrome*: Breast and ovarian cancer, as well as pancreatic cancer, can be caused by BRCA1 or BRCA2 mutations, especially in BRCA2. This mechanism explains between 17% and 19% of cases of hereditary pancreatic cancer (Moran et al. 2012).
(b) *Hereditary Non-polyposic Colorectal Cancer or Lynch Syndrome (HNPCC)*: Due to microsatellite instability (MSH2, MSH6, MLH1, PMS2, and EPCAM genes), persons with Lynch syndrome are more likely to develop colorectal cancer without polyps or other site neoplasia, particularly pancreatic cancer.
(c) *Familial Adenomatous Polyposis (FAP)*: Polyps in the gastrointestinal system can evolve into malignant neoplasia if the APC gene is mutated, causing this condition. Since ampulla carcinomas may be misdiagnosed as FAP, the link between FAP and pancreatic cancer remains unclear (Capasso et al. 2018; Giardiello et al. 1993).
(d) *Peutz-Jeghers Syndrome (PJS)*: A hamartomatous polyposis syndrome is characterized by STK11/LKB1 gene mutations, which can lead to gastrointestinal neoplasia and various malignancies, such as pancreatic cancer (Capasso et al. 2018).
(e) *Hereditary Pancreatitis (HP)*: Hereditary pancreatitis can be diagnosed in 80% of cases by identifying a PRSS1 gene mutation, which causes acute pancreatitis in children and can lead to a praecox pancreatic failure (Capasso et al. 2018). The pancreatic chronic inflammation that causes pancreatic cancer onset is triggered by this chronic inflammation (Capasso et al. 2018). HP patients are more likely to get pancreatic cancer than the overall population, according to some researchers (Capasso et al. 2018).

Environmental risk factors are

(a) *Use of tobacco*: Smoking is the greatest environmental threat element for pancreatic cancer; the pathogenetic pathways include gene alterations (KRAS, p53) and chronic inflammation, which can generate cytokines and growth factors, leading to cellular transformation. Smoking is to blame for between 20% and 35% of incidences of pancreatic cancer (Capasso et al. 2018).
(b) *Consumption of alcohol*: Having more than three drinks a day increased the incidence of pancreatic cancer by a factor of 1.22–1.36. Repetitive inflammation (60–90% of chronic pancreatitis) is caused by the long-term effects of alcohol and its metabolites (Capasso et al. 2018).
(c) *Chronic pancreatitis*: Chronic pancreatitis shares molecular and anatomical similarities with pancreatic cancer. Chronic inflammation produces TNF, IL-6, IL-8, PDGF, TGF, and other cytokines that increase cellular proliferation and impair immune-scrutiny (Capasso et al. 2018). ROS causes DNA destruction, encouraging cellular change (Capasso et al. 2018). Chronic pancreatitis increases the chance of pancreatic cancer, regardless of sex, geography, or type (Capasso et al. 2018).
(d) *Obesity*: Some studies showed a 1.12 relative risk increase for each increase in 5 kg/m^2 BMI (Capasso et al. 2018). In obese patients, adiposopathy is a chronic adipose illness in which macrophages produce pro-inflammatory cytokines and there is hormonal imbalance: high leptin and low adiponectin (Capasso et al. 2018). Obesity since childhood increases pancreatic cancer risk (Capasso et al. 2018). Red meat and fatty diets may contribute to the pathophysiology (Capasso et al. 2018).
(e) *Diabetes mellitus*: Glucose intolerance or diabetes affects about 80% of pancreatic cancer patients. These disorders are clearly linked, but it is vital to explain the connection. Diabetes is present in the vast majority of patients with pancreatic cancer during the course of the detection of tumour, supporting the theory that diabetes is caused by neoplasia in the pancreas (Capasso et al. 2018).

10.3 Treatment of Pancreatic Cancer: Therapeutic Challenges

Fifty to eighty percent of PDAC tumour volume is composed of stroma, making it one of the malignancies with the highest stroma content. The PDAC stroma promotes tumour growth and metastasis, acts as a physical obstacle to drug administration, and is extremely resilient to traditional therapy (De Sousa Cavalcante and Monteiro 2014; Thomas and Radhakrishnan 2019; Bulle and Lim 2020). Immune avoidance or limitation of immune surveillance is another characteristic of the pancreatic cancer tumour microenvironment; T regulatory cells (Treg), tumour-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs) make up the microenvironment of PDAC and impede the ability of CD8+ T cells to recognize and eliminate tumours (Martinez-Bosch et al. 2018).

Pancreatic cancer patients face additional difficulties in their treatment since early detection is difficult due to the lack of biomarkers and effective diagnostic radiological procedures (Kunovsky et al. 2018; Meleady et al. 2020; Montemagno et al. 2021). On the existing imaging technique, even if PDAC is believed to be identified at an initial stage, subclinical metastases may already be present. Theranostic nuclear imaging is expected to become more accurate in the future (Montemagno et al. 2021).

10.3.1 Surgery and Chemotherapy

twenty to thirty percent of locoregional illness patients can be cured by surgery (Riall and Lillemoe 2007). The median overall survival (OS) is 54.4 months if surgery is preceded by adjuvant dose-attenuated modification of folinic acid, fluorouracil, irinotecan, and oxaliplatin (mFOLFIRINOX) chemotherapy (Conroy et al. 2018, 2011). There is an 11.1-month median overall survival (OS) for patients with metastatic disease who receive the FOLFIRINOX combination of leucovorin, 5-FU, irinotecan, and oxaliplatin. The fittest patients receive FOLFIRINOX in adjuvant and advanced settings. After data from mFOLFIRINOX, ESPAC-4 had the best adjuvant OS outcomes. 722 patients were indiscriminately allocated to obtain adjuvant gemcitabine alone or GEM/CAP. Gemcitabine alone had a mortality rate of 0.82 (95% CI, 0.68–0.98), while GEM/CAP patients had a mortality rate of 28.0 months (95% CI, 23.5–31.5) and 25.5 months (95% CI, 22.7–27.9). Metastatic PDAC was treated with gemcitabine and nab-paclitaxel by MPACT. With nab-paclitaxel-gemcitabine and gemcitabine, the median survival time was 8.5 months (HR for death, 0.72; 95% CI, 0.62–0.83) (Von Hoff et al. 2013). In less fit patients, single-agent gemcitabine is still used as adjuvant and metastatically. These patients need novel medicines to improve survival.

10.3.2 Immunotherapy

Immune checkpoint inhibitors (ICI), such as anti-CTLA4 and anti-PD-1 ligand 1, are one of the most significant cancer therapeutic developments in the past decade. PDAC patients have trouble making immunotherapy progress. Ipilimumab (anti-CTLA4) was investigated in PDAC after success in melanoma. Seven patients with localized PDAC and 20 with metastatic disease got ipilimumab in a 2010 phase II trial. Opinionated (Royal et al. 2010). In a phase II trial, 32 patients with metastatic PDAC didn't respond to PD-L1 antibody durvalumab (O'Reilly et al. 2019). In a phase II study utilizing PD-L1 inhibitors like durvalumab and tremelimumab for 4 cycles, subsequently durvalumab continuation for 1 year, the ORR was 3.5% (Emens and Middleton 2015). According to current research, cytotoxic therapy may boost ICI's activity and efficacy in some cancers (Renouf et al. 2018). Gemcitabine, NAB-paclitaxel, durvalumab, and tremelimumab achieved 100% disease control in a Phase II trial for metastatic PDAC (Renouf et al. 2020). Gemcitabine and

nab-paclitaxel with or without durvalumab and tremelimumab had no influence on progression-free survival (PFS), overall survival (OS), or response rate (Marabelle et al. 2020) Pembrolizumab therapy for MSI-H/dMMR solid tumours was FDA-approved as tissue agnostic in 2017. PDAC had an ORR of 18.2% and median PFS and OS of 2.1 months (Geller et al. 2017) in the KEYNOTE-158 study among 233 patients with severe non-colorectal cancer. 0.8–2.0% of PDAC patients have MSI-H/dMMR (Yu et al. 2021). Compared to MSI-H cholangiocarcinoma (40.9%), small intestine (42.1%), gastric (45.8%), and endometrial cancers (57.1%), this trial's ORR for MSI-H PDAC was only 18.2% (Luchini et al. 2021). One of the earliest immunotherapies for PDAC was GVAX, a heterogeneous whole-cell vaccination consisting of two irradiated allogeneic PDAC cell lines engineered to release GM-CSF (Jaffee et al. 2001). GVAX showed some promise in phase I and II research with cyclophosphamide (Jaffee et al. 2001; Laheru et al. 2008). Ipilimumab and GVAX were investigated in a phase 1B trial on 30 previously treated patients with severe PDAC (Le et al. 2013). The median overall survival (OS) for patients receiving GVAX/ipilimumab was 5.7 months, but the median OS for those receiving ipilimumab alone was only 3.6 months. Combined GVAX and anti-PD-1 antibody treatment are being tested in a number of PDAC trials (NCT02451982; NCT02648282).

The comparative failure of immunotherapy in the management of PDAC patients is not completely understood; however, this may be attributed in certain manner to the microbiome of the patient that inhibits the effectiveness of immunotherapy.

10.4 Role of Human Microbiome in the Development of Pancreatic Cancer

Inflammation, immunological suppression, and microbial metabolites that de-regulate host genomic constancy all have a part in the cancer development, and the microbiota can influence all of these factors (Fig. 10.1) (Goodman and Gardner 2018; Hanahan and Weinberg 2011; Scott et al. 2019).

Pathogens, damaged cells, and toxic substances all trigger inflammation as a defence mechanism for the body (Shacter and Weitzman 2002; Coussens and Werb 2002; Chen et al. 2018). It removes harmful stimuli and initiates the healing process. Inflammation is one of the primary causes of pancreatic cancer, namely PDAC. Chronic pancreatitis causes exocrine and endocrine destruction that leads to a series of necrosis and fibrosis proceedings that are facilitated by pancreatic stellate cells (PSCs) (Witt et al. 2007). PSCs are exocrine cells, primarily acini, involved in tissue healing and the secretion of digestive enzymes (Omary et al. 2007). Not only are pancreatic cells sensitive to inflammatory signalling, but inflammatory alterations also affect the tumour microenvironment, particularly cancer-associated fibroblasts (CAFs). CAFs are associated with the release of extracellular matrix and other inflammatory components, constituting a major percentage of the pancreatic tumour microenvironment. In essence, CAFs are distinct PSCs that perpetuate disease and affect therapy resistance (Ohlund et al. 2017). CAFs are capable of secreting a

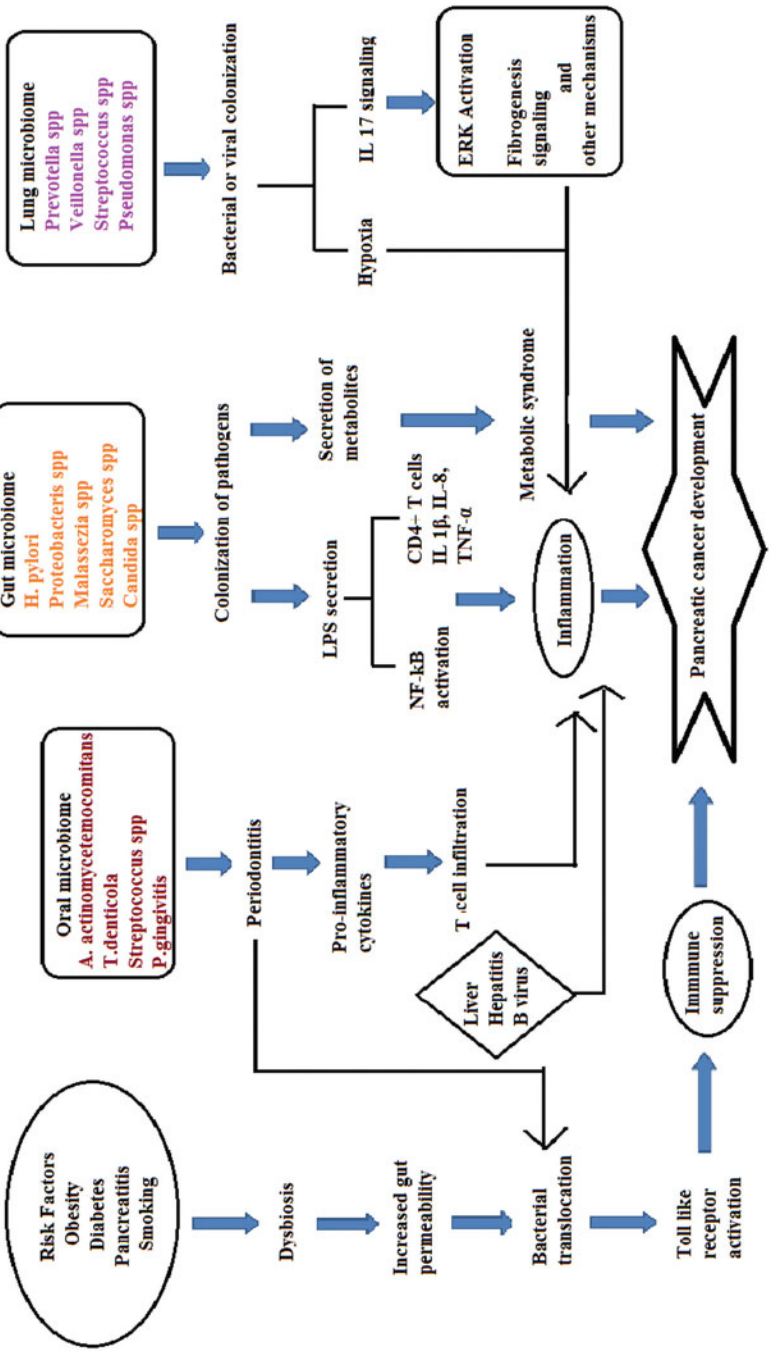

Fig. 10.1 Role of microbiome in the development of pancreatic cancer

variety of inflammatory signals, including interleukins, chemokines, and inflammatory factors (Domen et al. 2021).

Inflammation, via Ikβ Kinase 2 or COX2, has been linked to the development of PDAC in P53-deficient pancreatic cells (Swidnicka-Siergiejko et al. 2017). One of the pro-inflammatory cytokines released by the tumour microenvironment is IL-1α (interleukin 1α), a cytokine that promotes KRAS activation in this disease. Myeloid cells are recruited by KRAS mutant cells to release IL-6, which promotes the development and progression of the disease by activating STAT3, a well-known gene driving pancreatic cancer (Tjomsland et al. 2013). Microenvironmental CAFs are generally diverse and perform either inflammatory or myofibroblastic activities (Steele et al. 2021). According to research, myofibroblastic subset, although not directly signalling for inflammation markers, can indirectly enhance the CAF population, leading to an increase in the CAF population and an expansion of regulatory cells, which can result in immune evasion (Steele et al. 2021).

Ochi et al. revealed that the pancreatic cancer tumour microenvironment is overexpressed with toll-like receptors (TLRs) such as TLR4 and TLR7 which are well-recognized members of the family of pattern recognition receptors (PRRs) (Ochi et al. 2012a, b). When pathogen-associated molecular patterns (PAMPs) are activated, they can cause an inflammatory response in the body by triggering the PRRs in the body's immune system. In mice, stimulation of TLRs leads to pancreatitis and synergize with KRAS to promote pancreatic cancer. Both the NF-B and the MAPK pathways can be inhibited to counteract TLRs' carcinogenic activities (Ochi et al. 2012b) and so prevent their pro-cancerous consequences. The pancreatitis-protective effects of TLR deficiency are also shown in mice. TLR4 and TLR7 suppression prevents pancreatic carcinogenesis in KC mice (Ochi et al. 2012a, b).

Inflammation influences PDAC regulation and PanNET development. In a retrospective analysis, Gaitanidis et al. found that neutrophil-to-lymphocyte ratio does not predict metastatic potential, while platelet-to-lymphocyte ratio reflects progression of the disease and lymphocyte/monocyte ratio suggests cancer return following surgery (Gaitanidis et al. 2018). Interleukin-1 (IL-1), tumour necrosis factor alpha (TNF-α), and interleukin-6 have toxic effects on PNETs such as gastroenteropancreatic neuroendocrine tumours (Cigrovski Bervkovic et al. 2014). Serum levels of IL-2, TNF-α, or IL-6 are used to distinguish between GEP NETs that function and those that do not (Cigrovski Bervkovic et al. 2014). PNET tumours have been discovered to exhibit inflammatory alterations although the immunological landscape of the PNET tumour microenvironment is yet unknown. Neutrophils and mast cells infiltrate the immune system in great numbers (Cives et al. 2019).

Chronic pancreatitis patients are 13 times more likely to develop pancreatic cancer than those with autoimmune pancreatitis, which has a 40% lifetime chance of developing PDAC (Yadav and Lowenfels 2013). The degree of KRAS mutation and the degree of dysplasia in PanIN lesions, both of which correlate with the length of pancreatitis, rise in tandem, finally leading to the establishment of PDAC (Jin et al. 2013). This raises the possibility that repeated episodes of inflammation and the resulting genetic alteration are mutagenic and contribute to the development of PDAC.

Pro-inflammatory effects can be induced in distant organs via microbe-associated molecular patterns, as well as organ-specific effects. TLR4 and lipopolysaccharide (a PAMP) interact to activate cell survival pathways, resulting in carcinogenesis outside the gastrointestinal tract (Wang et al. 2012; Ertz-Archambault et al. 2017).

Carcinogenesis and microbiota could result in an increased immune reaction or a pro-tumorigenic response from bacteria. Several anticancer treatments stimulate the immune system via the microbiome of the gut, resulting in an increased immunological response. Zitvogel and colleagues observed, for instance, that cyclophosphamide treatment affects the intestinal mucous membrane, letting gut bacteria to spread to lymph nodes and spleen and activate certain immune cells (Viaud et al. 2013a). Cyclophosphamide, on the other hand, had no anticancer activities when fed to animals who had been treated with antibiotics or those that had no microorganisms (Viaud et al. 2013a; Iida et al. 2013). Similarly, Sivan et al. discovered that Bifidobacterium boosted mice's immunotherapy response, indicating that gut microbiota may stimulate the immune system (Sivan et al. 2015).

Immune response suppression can also come from the presence of microbiota. In mice models, eliminating microbiota from PDAC-carrying animals, but not from controls, lowers tumour protection (Pushalkar et al. 2018). Anti-PD1 inhibitors and microbiota-eradicating antibiotics had a synergistic anticancer effect (Pushalkar et al. 2018). Contradictory discoveries suggesting the microbiome can either stimulate or hinder the immune response to cancer imply that different cancer types may modify the gut and tumour microbiota content and effect treatment response, including ICI therapy.

Numerous biological and pathological processes, such as gene regulation, translation, cell proliferation, differentiation, stress resistance, tumour growth, and apoptosis, are dependent on microbial metabolites (Rowland et al. 2018). Numerous genes in colonic bacteria, such as *Faecalibacterium prausnitzii* and Eubacterium/Roseburia species, regulate metabolism and metabolize undigested dietary components (Louis et al. 2007). Short-chain fatty acids (SCFAs) are created by the saccharolytic fermentation of carbohydrates in a high-fibre diet (O'Keefe 2008; Scheppach 1994). Butyrate reduces colorectal cancer risk (Scheppach 1994; Wu et al. 2018).

However, when carbohydrates are reduced in the distal colon, proteolytic fermentation occurs, which leads to the production of inflammatory and carcinogenic metabolites, for example, ammonia, and other nitrogen-rich metabolites, phenols (Windey et al. 2012). Desulfovibrio vulgaris, a sulphate-reducing bacterium has been found to flourish in the presence of high protein and fat diets, which creates excess hydrogen sulphide that has been proved to be genotoxic (Kushkevych et al. 2021; Attene-Ramos et al. 2006).

Early starting events are characterized by KRAS mutations (Eibl and Rozengurt 2019). Nevertheless, oncogenic KRAS only is insufficient for the progression of aggressive PDAC. PDAC production necessitates added genetic abnormalities and environmental, dietary, and metabolic stresses, such as inflammation and obesity, to activate KRAS downstream effectors (Eibl and Rozengurt 2019). Other cancer models have established that a high-fat diet-induced dysbiosis enhanced KRAS-

driven intestinal carcinogenesis (Schulz et al. 2014). There is also evidence that obesity-related dysbiosis of the gut microbiota can affect obesity-related cancers, including PDAC (Djuric 2017; Li et al. 2020).

Several preclinical models have clarified how bacteria might cause carcinogenesis. Research by Gnansekaran et al. investigated the direct impact of *P. gingivalis* on the growth and proliferation of PDAC (Gnanasekaran et al. 2020). *P. gingivalis* infection increased PDAC cell growth, which correlates with intracellular survival. Hypoxia enabled *P. gingivalis* survive. *P. gingivalis* infection increased tumour growth in vivo, consistent with in vitro data. In an oral squamous cell model, *P. gingivalis* had an effect on squamous cell proliferation through the TLR2 receptor (Binder Gallimidi et al. 2015). *P. gingivalis*-induced PDAC cell proliferation was shown by Gnansekaran et al. to be independent of TLR2 signalling and linked to Akt signalling (Gnanasekaran et al. 2020).

Gut microbiota may affect PDAC tumour development and drug response. Pushalkar et al. employed genetically engineered PDAC animal models (KC and KPC) to show that intestinal bacteria can migrate into the pancreas (Pushalkar et al. 2018). Tumour growth and progression were delayed in germ-free and antibiotic-treated animals. Repopulating the gut microbiota with PDAC or *Bifidobacterium pseudolongum*-treated animals accelerated illness. Microbial ablation affected the tumour microenvironment, enhanced M1 macrophage differentiation, and decreased myeloid-derived suppressor cells. Microbial ablation increased PD-1 expression, enhancing the anticancer effects of PD-1 inhibition, suggesting the microbiota may be a therapeutic target in PDAC (Pushalkar et al. 2018).

It has been shown that antibiotic treatment reduces cancer incidence in a completely different mouse model (KrasG12D/PTENlox/+) by Thomas et al.. It took longer for xenografts to form in Nod-SCID mice with PDAC xenografts, and the tumours were smaller and grew slower after microbial depletion (Thomas et al. 2018).

Antibiotic therapy reduced tumour growth and metastatic burden in a PDAC animal model, which was connected to an increase in effector T cells in the tumour microenvironment (Sethi et al. 2018). In Rag1-KO mice, who lack fully mature T and B cells, antibiotic therapy had no effect on tumour size, suggesting that antibiotic anticancer action was not a direct cytotoxic effect and required adaptive immunity (Sethi et al. 2018). Aykut et al. (Aykut et al. 2019) investigated the involvement of the mycobiome in PDAC carcinogenesis. Mycobiome ablation by Amphotericin B reduced tumour progression and development in PDAC mice models, and repopulation with Malassezia boosted tumour growth. One mechanism by which microorganisms can cause tumours is through the complement cascade, which is set off when they attach to mannose-binding lectin (MBL) (Aykut et al. 2019).

Microbial dysbiosis changes the tumour immunological microenvironment and has been shown to influence PDAC tumour progression in animal studies. The microbiome has been discovered to be a possible therapeutic target; however, there are still considerable obstacles to overcome. Huge cohorts of real-world pancreatic cancer patients must be examined for the numerous groups that may influence positively or negatively to disease progression to reconcile conflicting

animal and human studies. Although long-term antibiotic use has been linked to a higher risk of cancer in humans (Petrelli et al. 2019), it has also been found to reduce tumour growth in mice (Pushalkar et al. 2018; Thomas et al. 2018; Sethi et al. 2018).

Antibiotic exposure before immunotherapy, but not contemporaneous antibiotic use, has been shown to impair clinical outcomes in some non-PDAC malignancies (Pinato et al. 2019), although the evidence for this is mixed and the subject of intense discussion continues to rage on (Hakozaki et al. 2020; Jin et al. 2019). A better understanding of microbiome dynamics and immune system interactions is needed to improve therapy.

10.4.1 Association of Pancreatic Microbiome with Pancreatic Cancer

Over 100 years ago, researchers detected bacteria in human tumours, but they were hard to define due to low biomass and sample contamination (Nejman et al. 2020). New analysis techniques have enhanced our understanding of these bacteria. Amplified 16S ribosomal RNA (rRNA) is sequenced to identify bacterial populations, while internal transcriber spacer (ITS) sections between rRNA component genes are sequenced to identify fungi (Kuczynski et al. 2012; Dollive et al. 2012). Researchers have found remarkable patterns in the microbiomes of healthy persons and PDAC patients using these methods.

Proteases in pancreatic juice and an alkaline pH make it impossible for most microbes to thrive (Maekawa et al. 2018). Using 16S rRNA fluorescent probes and qPCR, PDAC patients had 1000-fold more intrapancreatic bacteria than normal (Pushalkar et al. 2018; Dickson 2018). PDAC, pancreatic benign neoplasm, and healthy cohorts had different relative numbers of several taxa (Olson et al. 2017). Compared to intestinal microflora, several bacteria increased in PDAC patients' pancreas. If the variations in microbiome characteristics between benign and malignant pancreatic disease can be clearly established, then early detection, therapeutic effectiveness, and prognosis in PDAC may be improved with a larger cohort.

Pushalkar et al. evaluated 12 PDAC and 5 normal pancreatic samples (Pushalkar et al. 2018). FISH revealed that human PDAC samples had more bacteria than normal pancreas samples. 16S rRNA sequencing revealed that Proteobacteria, Bacteroidetes, and Firmicutes were prominent intratumoral phyla. Clade abundances showed that PDAC had a different bacterial composition than normal pancreatic (Pushalkar et al. 2018). Additional studies of PDAC microbiota showed a unique intratumoral bacterial profile (Nejman et al. 2020; Jeong et al. 2020; Rogers et al. 2017).

Gemcitabine proved useful in some individuals with advanced pancreatic cancer, but most developed drug resistance and failed treatment. Geller et al. (Geller et al. 2017) found Gammaproteobacteria in gemcitabine-resistant PDAC tissue samples and hypothesized that they could regulate gemcitabine sensitivity. Pushalkar et al. (Pushalkar et al. 2018) studied intratumoral microbiota in PDAC development and treatment response. In a longitudinal study of age-matched KC (p48Cre; LSL-KrasG12D) and wild-type mice, some bacterial communities were enriched

in KC mice, with *Bifidobacterium pseudolongum* being the most prevalent. These research showed how intratumoral microbiome affects cancer's natural history.

Once *H. pylori* infect human pancreatic cells, it may cause adenocarcinoma (Nilsson et al. 2006). A preclinical study (Takayama et al. 2007) found that *H. pylori* colonization in pancreatic cancer cells activated molecular pathways governing PDAC development and progression. Pancreas and gastroduodenal tissues had different Helicobacter subspecies. Fusobacterium colonization in PDAC patients is an independent predictive marker for significantly shorter survival (Mitsuhashi et al. 2015), in contrast to oral Fusobacterium, which reduces pancreatic cancer risk.

Researchers have discovered that the fungus (mycobiome) in PDAC samples differs from that seen in healthy samples, in a manner similar to the bacterial findings (Aykut et al. 2019). Fungal communities of PDAC patients were found to be distinct from those of healthy individuals when they studied the intrapancreatic mycobiome of 13 patients and 5 healthy individuals. Malassezia species were found to be overrepresented in PDAC samples (Aykut et al. 2019).

10.4.2 Association of Gut Microbiome with Pancreatic Cancer

In addition to intratumoral dysbiosis, other investigations have demonstrated that the patients with PDAC have a different microbiota in their gut than healthy people. Disturbances in the gut microbiome have the ability to control inflammation. Recent research shows that pancreatic cancer microbiome enhances adaptive and innate immune system responses, resulting in immune suppression and cancer evasion (Sexton et al. 2022). Helicobacter pylori and HBV are examples (Sexton et al. 2022). *H. pylori* is a documented bacterial carcinogen that promotes gastric cancer, however, its existence in PDAC patients is questioned and generally linked to initiating inflammatory processes (Sexton et al. 2022). Bacterial germs can cause inflammation by releasing lipopolysaccharide (LPS) chains, which activate NF-kB (Sexton et al. 2022). LPS causes acute pancreatitis, a precursor to pancreatic cancer, and causes CD4+ T-cells to secrete TNF-, IL-1, and IL-8 (Sexton et al. 2022). For the purposes of examining the microbiota of 85 pancreatic cancer patients and 57 healthy controls, faeces were collected and analysed prospectively by Ren et al. (Ren et al. 2017). The variety of intestinal microorganisms was dramatically reduced in PDAC patients. In comparison to healthy controls, the gut microbiota of patients with PDAC composed of considerably more Bacteroidetes and significantly fewer Firmicutes and Proteobacteria (Ren et al. 2017). Rogers et al. (Rogers et al. 2017) analysed 50 pancreaticoduodenectomy patients' faeces, pancreatic fluid, bile, and jejunal fluid for microorganisms. The faeces samples had less microbial variety than those from healthy people, and Klebsiella and Bacteroides were added (Rogers et al. 2017). Half and associates (Half et al. 2019) studied the faecal microbiota of 30 PDAC patients, 13 healthy people, and 16 non-alcoholic fatty liver disease patients (NAFLD). No variations in microbial diversity were found between groups, but PDAC patients had a larger ratio of Bacteroidetes to Firmicutes than controls,

consistent with Ren et al. (Ren et al. 2017; Half et al. 2019). Results from a small case-control research found no substantial difference in the duodenal mucosal flora between PDAC patients and healthy individuals (Mei et al. 2018). Studying duodenal fluid from patients with pancreatic cysts, PDAC patients, and healthy controls, researchers found that patients with PDAC had a considerably lower diversity of bacterial and fungal organisms than those with pancreatic cysts or healthy controls (Kohi et al. 2020). Patients with PDAC reported higher concentrations of Bifidobacterium and Ascomycota than healthy controls. Patients with pancreatic cysts and healthy individuals had similar microbial patterns in their duodenal fluid (Kohi et al. 2020). Interpretation and generalization of 16S rRNA amplification results are problematic because of the wide range of study sizes, designs, sample methods, and primers employed. In addition, numerous chronic disorders show a decrease in gut microbial diversity relative to healthy people (Aldars-García et al. 2021; Alamri 2021; Hrncir et al. 2021).

Over 100,000 viruses have been discovered in the gut microbiome, many of which have never been investigated before (Nayfach et al. 2021). Due to the close closeness of the pancreas and the liver, many studies have linked HBV exposure to pancreatic cancer (Hoefs et al. 1980). HBV is able to spread between the liver and the spleen because they share blood arteries and ducts. The pancreatic juice contains a marker for HBV infection, the Hepatitis B surface antigen (Hassan et al. 2008). Hepatitis predominantly affects the liver, but the gut microbiota has been linked to HBV infection, and individuals with HBV infection often have stomach mucosal ulcers (Xia et al. 2005). There is now just hepatitis infections connected to the development of pancreatic cancer, but because the microbiome contains over 100,000 viruses, additional research is needed to find a link between the two. Another factor in pancreatic illness is mycobiome, which contains fungus and yeast. The gut's mycobiome contains a significant proportion of the fungus species in the Candida genus. Multiple malignant pancreatic cysts were discovered in the pancreas of a 56-year-old immune compromised man (Bulajic et al. 2014). Further research revealed that the cysts were caused by the fungal infection Candidiasis, which mirrored pancreatic cancer (Seong et al. 2015). Increasing evidence suggests that immunocompromised patients with Candida infection have higher incidences of several malignancies, including pancreatic cancer (Sexton et al. 2022; Chung et al. 2017).

10.4.3 Association of Oral Microbiome with Pancreatic Cancer

A wide variety of microorganisms make up the oral microbiome, which is found in the mouths of people with pancreatic cancer. Lu and colleagues studied the microbiota of the tongue covering of 30 patients with pancreatic cancer and 25 healthy controls (Li et al. 2021). The bacterial makeup of the tongue covering differs greatly between PDAC patients and healthy controls. Haemophilus, Porphyromonas, Leptotrichia, and Fusobacteria may distinguish PDAC patients from healthy people (Li et al. 2021). Diverse bacterial species have been identified

as the distinguishing feature in various investigations (Li et al. 2021). It is possible that the inconsistency stems from variations in sample size, study design, or geographic location in the same way that research on gut microbiota has.

When Wei et al. conducted a retrospective investigation of saliva samples from pancreatic cancer patients; they found an increase in the bacteria Streptococcus spp. and Leptotrichia spp. (Sexton et al. 2022). These microbes were detected in the saliva of Asian individuals with pancreatic cancer. *Porphyromonas gingivitis* and *A. actinomycetemcomitans* have been associated with a greater risk of pancreatic cancer in a retrospective study of patients in the United States. It was revealed that there were no significant changes in the oral microbiomes of healthy individuals and those with PDAC among African-Americans. The socioeconomic hindrances observed in many African American populations were found to be a larger risk factor for African American women having pancreatic cancer, despite this lack of difference (Sexton et al. 2022). People over the age of 65 with periodontal disease (gum disease) had a higher risk of developing PDAC than those with other illnesses such diabetes or pancreatitis (Sexton et al. 2022). As a consequence of periodontal disease, periodontal tissue degrades rapidly, resulting in abscesses, infection, and tooth loss. Gram-negative anaerobic bacteria, such as *P. gingivalis* and *T. denticola* (Sexton et al. 2022), are frequently linked to periodontal disease. When oral bacteria enter the lower gastrointestinal system, it travels directly to the pancreas via the portal circulation of the lower gastrointestinal tract, which is still under investigation (Sexton et al. 2022).

10.4.4 Association of Lung Microbiome with Pancreatic Cancer

In spite of the deficiency of research demonstrating a direct relationship between lung microbiome and the emergence of pancreatic cancer, numerous processes known to take place within the lung microbiome have been linked to the disease. Interleukin-17 (IL-17) signalling activity was discovered to occur in patients with asthma and sarcoidosis and was linked to the colonization of harmful bacteria within the lungs (Sexton et al. 2022). As a result of increased inflammation and activation of ERK1/2 and fibrogenesis genes, IL-17 overexpression was observed to stimulate the progression of acinar–ductal metaplasia (ADM), intraepithelial pancreatic neoplasia (PanIN), and PDAC. Pancreatic cancer development can be influenced by changes in the lung microbiome as well as the status of the lungs following trauma or microbial disturbances. Hypoxic or hypoxaemic situations can be induced by a variety of bacterial or viral colonized lung illnesses, including as bronchitis, COVID-19, and/or pneumonia. As a result of the hypoxic tumour microenvironment created by limited vasculature, pancreatic cancer often develops with lower response rates and poorer overall survival as a result of hypoxia settings. HIF-1 hypoxia genes have been demonstrated to influence pancreatic carcinogenesis (Sexton et al. 2022).

10.4.5 Association of Skin Microbiome with Pancreatic Cancer

Though it appears implausible that the microbiome of the skin might contribute to the progression of pancreatic cancer, there is adequate indirect evidence linking the two. There is a decreased number of Gram-negative bacteria, particularly proteobacteria on the skin, according to recent 16S sequencing results (Sexton et al. 2022). Typically, the skin contains an enormous number of Gram-positive bacteria. Proteobacteria were discovered to be more prevalent in PDAC tumour patients than in healthy persons (Sexton et al. 2022). Aykut et al. observed that Malassezia species were abundant in PDAC tumours, but Candida and Saccharomyces species were significantly reduced. One type of fungus known as Malassezia species was shown to play a role in pancreatic cancer progression by interacting to the mannose-binding lectin (MBL) and triggering a complement cascade to elude the immune system (Sexton et al. 2022). In pancreatic cancer tissues, it is above 3000 times more prevalent than normal tissue. This fungus is often present in the skin microbiome, particularly on the scalp, and adds to dandruff and seborrheic dermatitis (Sexton et al. 2022). Proteus spp. was also discovered to be substantially elevated in tumours of pancreatic cancer (Sexton et al. 2022). Proteus species were discovered in 90% of cutaneous and urinary tract infections, have a high pathogenicity potential, and are associated with obesity (Sexton et al. 2022).

10.5 Modulation of Gut Microbiome as a Promising Therapeutic Strategy for Pancreatic Cancer

Pancreatic cancer and the human microbiome, particularly gut microbiota are closely linked, thus it is possible to alleviate symptoms by altering the gut microbiome with products and treatments such as probiotics (Hill et al. 2014), prebiotics (Gibson et al. 2017), synbiotics (Swanson et al. 2020), postbiotics (Salminen et al. 2021), and FMT (Cammarota et al. 2017). Gut microbiome–pancreatic cancer interactions can be improved by a better knowledge of the causal link between these two.

10.5.1 Potential Use of Prebiotics for Treatment of Pancreatic Cancer

"A non-digestible food element that positively affects the host by selectively encouraging the growth and activity of one or a restricted number of bacteria in the colon, and thereby enhances host health" (Abdul Rahman et al. 2021) was the original description of prebiotics. Lately the definitions of prebiotics were updated by the ISAPP consensus statement (2016) as "a substrate that is selectively utilized by host microorganisms conferring a health benefit", and include conjugated linoleic acids, polyunsaturated fatty acids (PUFAs), oligosaccharides such as galactooligosaccharides (GOS), fructooligosaccharides (FOS),

xylooligosaccharides, mannanoligosaccharides, inulin, and human milk (Abdul Rahman et al. 2021; Zhang et al. 2022).

Prebiotics are quickly utilized by intestinal microbes, resulting in metabolic products like short-chain fatty acids (SCFAs), such as acetate, butyrate, and propionate which are critical to intestinal health. The liver and muscle use propionate and acetate for gluconeogenesis and energy production, respectively, while colonocytes use butyrate as their principal energy fuel (Abdul Rahman et al. 2021).

Prebiotics may function independently of probiotics and exert direct effects on the gut. Prebiotic oligosaccharides that imitate microvillus glycoconjugates can inhibit pathogen attachment to epithelial cells by binding to the bacterial receptor, therefore lowering pathogen colonization (Abdul Rahman et al. 2021).

Chitosan oligosaccharides diminished the extremity of pancreatic damage in mice by lowering oxidative stress and modifying the intestinal flora prior to severe acute pancreatitis (SAP) induction (Zhang et al. 2022). In a randomized, double-blind clinical trial of patients with SAP, correlations between prebiotic fibre intake and length of hospital visit, extent of nutrition therapy, acute phase reaction, and general problems were found (Zhang et al. 2022). Inulin type fructans are regarded to have an important effect in the prevention of AP and Type 1 Diabetes (Zhang et al. 2022). Increasing the synthesis of colonic SCFA by supplementing NOD mice with low-methoxyl pectin reduced T1DM in the animals (Zhang et al. 2022). Human milk oligosaccharides were also found to influence T1DM in NOD mice in a different investigation, with similar outcomes (Zhang et al. 2022). Glycaemic management in children with type 1 diabetes (T1DM) can be improved with the use of prebiotics (oligofructose-enriched inulin) (Zhang et al. 2022).

A rising number of studies are looking into the potential benefits of prebiotics for treating pancreatic cancer. There are few research in PDAC that have interesting findings (Sobocki et al. 2021). Trivieri et al. used a pancreatic cancer gene expression dataset (GSE16515) to evaluate RSD's effect on tumour tissue miRNA expression profiles. High-compound diets decreased 19 miRNAs gene expression relative to the control group. The authors employed creativity pathways analysis to predict the biological roles of RSD-fed mice's miRNA genes (Sobocki et al. 2021). Researchers found that mice fed RSD had a lower level of gene expression than those fed a control diet, suggesting that RSD may inhibit the formation and spread of tumours and other malignancies. The RSD-fed mice also had an increased expression of genes involved in carbohydrate production, glucose metabolism dysfunction, and cancer cell death. On top of that, IPA analysis of the PDAC signalling network revealed increased expression of TGFBR2, AKT, and other genes in mice given RDS. Analysis of TCGA data was used to examine the relationship between 19 distinct miRNAs and the prognosis of PDAC patients (Sobocki et al. 2021). MiRNA-375, miRNA-148a-3p, miRNA-125a-5p, and miRNA-200a-3p were all shown to be strongly linked with PDAC prognosis in mice fed RSD. These genes are connected with significantly better results and longer overall survival, which supports the use of RSD in patients with pancreatic cancer (Sobocki et al. 2021). In order to avoid bias from the indirect conclusion, it must be ensured that this conclusion is tested on a large and diverse population of patients. Additionally, the

metabolomic composition in pancreatic tissue can be altered by the RSD diet, according to yet another study (Sobocki et al. 2021).

RNA-Seq research found 25 genes dysregulated in RSD-fed mice vs. control mice. LC-MS analysis identified six dysregulated blood metabolite levels. These genes were linked by a bioinformatics analysis to processes as diverse as insulin receptor signalling and circadian rhythm signalling, as well as cancer drug resistance, cell death and survival, gene expression, and neurological diseases. As glutamine levels increased, acetylcarnitine, arginine, aspartic acid, hypoxanthine, inosine, and xanthine levels decreased. The purines hypoxanthine, inosine, and xanthine serve as a "fuel" for enhanced cancer metabolism, which is well-known (Sobocki et al. 2021).

Panebianco et al. found that RSD-fed mice had decreased blood purine levels, which may inhibit cancer cell growth. This study doesn't reveal tumour purine levels. In RSD-fed rats, glutamine absorption and utilization by tumours were restricted because to high blood glutamine levels and low glucose availability (Sobocki et al. 2021). More research is required to better realize the links between RSD, the tumour microenvironment, and blood components. Clinical study could disclose how RSD impacts PDAC patients' metabolome, gene expression, and survival.

10.5.2 Probiotics for Prevention and/or Treatment of Pancreatic Cancer

According to the World Health Organization, "live microorganisms that, when administered in suitable proportions, impart a health benefit on the host" are probiotics. Lactobacillus spp. and Bifidobacterium spp. are the two utmost typical lactic acid bacteria (Hill et al. 2014). Anaerobic Gram-positive Lactobacillus species are considered "good bacteria" because they break down carbohydrates and compete with pathogens in the gut. Fermented foods like kombucha, kimchi, and raw, unfiltered apple cider vinegar all contain probiotics (Davis 2016). Yogurt, cheese, milk, juices, and smoothies are other good sources of probiotics.

More and more research show that probiotics can reduce the risk of developing cancer in humans through the enhancement of the body's immune system, decrease in levels of oxidative stress and increase in gut microbial diversity as well as improve intestinal barrier integrity (Abdul Rahman et al. 2021). The use of probiotics in cancer patients, particularly those who are receiving immunosuppressive medicines, has raised concerns. It is possible that resistant genes could be transmitted from the bacteria to the resident microbiota, resulting in an increase in antimicrobial resistance (Abdul Rahman et al. 2021).

Bacterial translocation can be exacerbated by gut dysbiosis, which alters intestinal barrier function, resulting in chronic pancreatitis and pancreatic cancer. Probiotics have been proven to stabilize the intestinal barrier in a number of animal experiments (Abdul Rahman et al. 2021). It has been shown that using Lactobacillus plantarum in nasojejunal tube feeding decreases the threat of pancreatic sepsis in

patients with acute pancreatitis, and those patients require fewer surgical treatments than control patients (Abdul Rahman et al. 2021).

Studies have shown that taking probiotics can diminish the danger of cancer formation and recurrence in a wide range of cancer types, including colorectal, breast, and bladder (Abdul Rahman et al. 2021). Lactobacillus casei Shirota, for example, has been indicated by Matsuzaki et al. to reduce chemically induced carcinogenesis and to counteract metastasis in transplantable tumour cells. To reduce tumour growth and improve survival in tumour-induced sarcoma 180 mice, *L. casei Shirota* was administered intraperitoneally (Abdul Rahman et al. 2021). Several cytokines, including interferon-gamma (IFN-γ), interleukin 1 beta (IL-1β), and tumour necrosis factor-alpha (TNF-α), were responsible for this process.

By altering MAPK/ERK signalling, Aspergillus oryzae was found to have anti-tumour properties (Sexton et al. 2022). The ferrichrome complex, an iron molecule found in probiotics, was also found to suppress pancreatic cancer (Sexton et al. 2022) and was effective against 5-FU-resistant cancer cells. In addition to promoting pancreatic cancer cell death, probiotics also have a prophylactic effect against the disease. Probiotics have been shown to lower inflammation, which can lead to pancreatitis and pancreatic cancer in some cases (Sexton et al. 2022).

Anti-tumour efficacy of gemcitabine in animals with pancreatic cancer could be improved by using the probiotic Lactobacillus (Zhang et al. 2022). The findings of this study are bolstered by a recent study that found that probiotics can lessen the negative effects of gemcitabine by restoring a healthy microbiome (Zhang et al. 2022). As a result, NK cells that had been pre-treated with probiotics were able to kill and differentiate pancreatic tumours in humanized-BLT mice (Zhang et al. 2022). Anti-tumour actions of probiotics may be mediated by the MAPK-p38 and TGF-b signalling pathways, according to this study (Zhang et al. 2022). Most published studies on the benefits of probiotics in the treatment of pancreatic cancer have been undertaken in animal models. For the treatment of pancreatic cancer patients, there is currently insufficient information to make any firm conclusions on the effects of probiotics. Future preclinical and clinical studies are needed to determine whether or not probiotics can help delay or stop the progression of pancreatic cancer. Next-generation sequencing and bioinformatics technologies are used to identify next-generation probiotics (Sobocki et al. 2021). Among the most promising new probiotic strains are *Akkermansia muciniphila, Prevotella copri*, *Faecalibacterium prausnitzii*, *Bacteroides thetaiotaomicron, Parabacteroides goldsteinii*, *Bacteroides fragilis*, *Christensenella minuta*. In terms of butyrate-producing bacteria, next-generation probiotic *Faecalibacterium prausnitzii* is a critical component of a healthy digestive system (Sobocki et al. 2021). According to a recent study by Zhou et al. PDAC patients have significantly increased level of *Proteobacteria phylum* (particularly Gammaproteobacteria) and drastically reduced level of butyrate-producing bacteria such as *Faecalibacterium prausnitzii*, *Eubacterium rectale*, *Roseburia intestinalis* (Zhou et al. 2021). As an important Gram-negative bacterium in the human body, *Akkermansia muciniphila* performs a wide range of functions, including maintaining intestinal immunity, regulating the production of cytokines, and combating infections (Derrien et al. 2004; Jayachandran et al. 2020).

The proliferative activity of rat pancreatic islet cell tumour cells (INS-1) was observed to be decreased by live *A. muciniphila* (Liu et al. 2020). Not only in relation to pancreatic cancer, but as a whole, there are not enough studies on next-generation probiotics. Nonetheless, they may provide cancer patients with new insights.

10.5.3 Postbiotics for Treatment of Pancreatic Cancer

Postbiotics are the soluble by-products and metabolites produced by gut microbiota that exert biological activities on the host (Abdul Rahman et al. 2021). A postbiotic was not officially defined as such until 2021, when the ISAPP stated that it is "a preparation of inanimate microorganisms and/or their components that bestow a health benefit on the host" (Salminen et al. 2021). Short-chain fatty acid (SCFA) is a well-known example, as it is created during probiotic fermentation. Postbiotics provide an effective and safer alternative to the intake of live microorganisms (Abdul Rahman et al. 2021). In addition to protecting the intestinal epithelium, postbiotics possess specific cytotoxicity against tumours.

Postbiotics may be the upcoming frontier in microbial therapies and functional foods, as evidence is mounting showing they provide a wide range of health advantages (Nataraj et al. 2020).

Pancreatic cancer may benefit from the use of the probiotics indicated above. There have been multiple meta-analyses that show the benefits of probiotics in a variety of medical disorders (Derwa et al. 2017; Szajewska and Kołodziej 2015). Probiotic therapy may have a positive effect on animals with pancreatic cancer; however, a meta-analysis has not yet been done to demonstrate this effect in humans.

Safety of probiotics in patients at high risk (such as those with acute pancreatitis) has been called into question by the results of single studies (Kothari et al. 2019); hence, some researchers are exploring using postbiotics in place of probiotics to treat these individuals. In contrast, the field of postbiotics is advancing rapidly, yet it is still a relatively unexplored one (Fong et al. 2020). Postbiotics as a concept has not yet been fully defined. SCFA, phenols, vitamins, supernatants, exopolysaccharides, enzymes, bacterial lysates, and cell wall pieces are all examples of beneficial bacterial metabolites that can be included in postbiotics (Zółkiewicz et al. 2020). Probiotics, on the other hand, appear to have a greater detrimental effect on the microbiota of patients than bacterial metabolites. For example, the intestinal microbiota may secrete "beneficial" compounds, such as postbiotics (Sobocki et al. 2021).

Lactobacillus casei ATCC334-derived ferrichrome has been found to suppress pancreatic cancer cell proliferation (Zhang et al. 2022). Some researchers have suggested that postbiotics may have an anti-inflammatory effect, restore the integrity of the gut barrier, or exert selective cytotoxicity against pancreatic cancer cells (Fong et al. 2020). However, there are few studies that have examined the role of postbiotics in pancreatic cancer patients. There are many examples of this first postbiotic action in Lactobacillus, such as the production of P40, which prevents

epithelial gut barrier breakdown (inducing inflammation in the body) (Wang et al. 2014; Gao et al. 2019). Supernatants from cultures of Bifidobacterium breve CNCM I-4035 (Bermudez-Brito et al. 2013) or other Lactobacillus cases, *Lactobacillus acidophilus*, *Lactococcus lactis*, and *Saccharomyces boulardii* have been shown to have anti-inflammatory activities (De Marco et al. 2018).

There is still a lot to learn about postbiotics in cancer treatment. In preclinical and clinical settings, it is extremely difficult to isolate the therapeutic molecule and characterize its safety profile because of the wide variety of metabolites that exist. This is an area that is anticipated to continue to grow as a cancer treatment in the future.

10.5.4 Synbiotics: A Prospective Therapeutic Approach for Pancreatic Cancer

Prebiotics and probiotics are combined in the term "synbiotics" (Abdul Rahman et al. 2021). ISAPP consensus declaration issued in 2019 defines symbiotic as "a mixture including living microorganisms and substrate that is preferentially utilized by host microorganisms that gives a health advantage" (Swanson et al. 2020). Adding synbiotics to neoadjuvant oesophageal cancer chemotherapy has been shown to increase the gut microbiome and lessen the negative effects of chemotherapy (Abdul Rahman et al. 2021).

Due to a small lifespan of probiotics in the gastrointestinal tract, their use may be necessary. In spite of this, there are not many researches describing synbiotics in PDAC. Studies on acute pancreatitis may help us form some conclusions.

Enteral feeding with synbiotics enhanced intestinal barrier function as well as reducing organ dysfunctions in individuals with SAP (Zhang et al. 2022). Vitamin C, magnesium, and albumin levels were increased in patients with CP who were given synbiotics containing *Bifidobacterium bifidum*, *Lactobacillus acidophilus*, *Lactobacillus Rhamnosus*, and fructooligosaccharides. The patients' nutritional status was not affected by the supplementation of these synbiotics (Zhang et al. 2022). Septic complications, hospitalization time, and antibiotic use were all reduced significantly in patients undergoing CP pancreatic surgery when synbiotic supplements comprising *Bacillus mesentericus*, *Lactobacillus sporogenes*, *Streptococcus faecalis*, *Clostridium butyricum*, and fructooligosaccharides were administered (Zhang et al. 2022). The synbiotic supplementation of individuals with T1DM improved fasting blood glucose, hemoglobin A1c, insulin, hypersensitive C-reactive protein and total antioxidant capacity in a randomized, controlled, double-blind, and placebo-controlled study (Zhang et al. 2022).

A randomized, double-blind trial employed beta-glucan, inulin, pectin, and resistant starch as prebiotics, along with four lactobacilli preparations (containing 10^{10} CFU). Fewer patients in the synbiotic group had systemic response syndrome (SRS) following recovery (Sobocki et al. 2021). Non-significant results included lower rates of multi-organ failure, septic complications, and mortality in the group receiving synbiotics. Despite the fact that chronic pancreatitis has been linked to the

development and progression of pancreatic cancer, any inferences about PDAC from this illness may be tainted by significant bias. PDAC-specific investigations are urgently required.

10.5.5 Faecal Microbiota Transplantation for Treatment of Pancreatic Cancer

Stool from a healthy donor can be transplanted into intestine of another person in the form of faecal microbiome; this procedure is called faecal microbiome transplantation (FMT) (Gupta and Khanna 2017). FMT to both antibiotic-treated and GF mice resulted in a worsening of the acute pancreatitis (AP) condition (Zhu et al. 2019). However, faeces from heparanase-transgenic mice were transferred to those of wild-type mice, and the illness in both groups became worse (Lei et al. 2021). An acute necrotizing pancreatitis (ANP) mouse diet and Western-type diet have also been found to be similar (van den Berg et al. 2021). However, one case study found that FMT was a successful treatment for MSAP patients (Hu et al. 2019). In order to determine the precise role that FMT plays in the beginning of pancreatitis, further animal and human investigations are required. Female NOD mice have a 1.3–4.4 times greater incidence of T1DM in particular pathogen-free NOD mice. Increased testosterone, metabolomic alterations, decreased islet inflammation, and decreased autoantibody synthesis were all observed following the gavage transfer of gut microbiota from adult males to females (Markle et al. 2013). MyD88-deficient NOD mice were shown to have a delayed onset of diabetes and a lower incidence of insulitis after faecal transplantation into wild-type female NOD/LtJ mice (Zhang et al. 2022). NOD animals exposed to antibiotics in early life had an increased risk of developing T1DM, whereas maternal cecal microbiota transfer returned the risk to baseline levels in the NOD mice (Zhang et al. 2022). Randomized clinical trials in 2021 indicated that FMT prohibited the progress of type 1 diabetes in humans (Zhang et al. 2022). FMT experiments in humans for T1DM are ongoing (NCT04124211; NCT04749030).

A Phase I clinical trial (NCT04975217) for pancreatic cancer is currently conducted to investigate the therapeutic benefit of FMT. The overall rationale for using FMT is because (1) pancreatic tumours contain bacteria like *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and Fusobacterium, (2) different bacterial species in healthy people's gut microbiomes decrease these unsafe bacteria, and (3) to achieve remission from pancreatic cancer, these cancer-causing microorganisms must be eradicated. Further study is needed to ascertain the microbiome profile of a healthy donor and to discover atypical bacteria that may be present in the donor's microbiome, however faecal transplantation does appear to be a successful treatment for pancreatic cancer. Drug-resistant bacterium was detected in a recently published report, and it was found to be the cause of a recent fatality (Sexton et al. 2022). Although the donor was uninjured by the drug-resistant bacterium, its transplantation into an immune compromised individual was harmful

(Sexton et al. 2022). Except for the research described previously, very few have looked into the use of FMT in pancreatic cancer.

10.5.6 Preventive Approaches for Pancreatic Cancer

A large proportion of the people are expected to have periodontal ailment and/or to have risk factors for this illness, such as tobacco use, age, anxiety and poor oral cleanliness, and diabetes (Sexton et al. 2022). There is some evidence to propose that poor periodontal health may have a role in the emergence of pancreatic cancer, as previously described. Socioeconomic disadvantages, such as a lack of dental care, the availability of unhealthy food at lower prices than healthy food, an increase in cigarette usage, and living in countryside areas, make it challenging for many people to maintain excellent oral health. It will be easier to address the gaps that exist within susceptible areas if healthcare professionals and the general public have a better grasp of the dangers of inadequate oral health and pancreatic cancer progression.

As mentioned earlier pancreatic cancer has been linked to an imbalance in the body's microbiome, which can be caused by a poor diet. Knowledge and understanding about the dangers of eating a diet high in processed foods and sweets may help keep the microbiota in check. Bacteria within the gut microbiome can be reversed by increasing ingestion of fermented foods, such as yoghurt, high fibre, and whole meals.

As a result of this, smoking has been shown to affect the human microbiome and contribute to the growth of dangerous bacteria in the stomach (Gui et al. 2021). Nicotine has been shown to elevate the pH of the gastrointestinal system through the actions of organic compounds which are volatile in nature (VOCs), aldehydes and polycyclic aromatic hydrocarbons (PAHs) found in cigarettes (Gui et al. 2021). When other risk factors are taken into account, smoking doubles the chance of pancreatic cancer. Humans' microbiomes are also affected in this way. As well as preserving the microbiome from pathogenic impacts on one's body, avoiding nicotine and tobacco products would help reduce several health risks of tobacco usage.

A healthy microbiota can be maintained by taking supplements and vitamins, as numerous studies have shown. When it comes to maintaining a healthy gut microbiome, B-vitamins have been demonstrated to reduce pathogenic and competitive bacterial species while concurrently sustaining the host–gut microbiota symbiotic relationship (Uebanso et al. 2020). There is evidence that supplementing with vitamin D helps older men's gut microbiome remains healthy (Thomas et al. 2020). Firmicutes and butyrate-producing bacteria increased in abundance as a result of increased vitamin D levels (Thomas et al. 2020); this was confirmed by a diversity analysis using 16S rRNA bacterial sequencing. Butyrate has been demonstrated to decrease pancreatic cancer invasion and increase the sensitivity of pancreatic cancer cells to histone deacetylases and gemcitabine (Farrow et al. 2003; Natoni et al. 2005; Chen et al. 2019).

10.5.7 Microbiome modulation: Impacts in Pancreatic Cancer

10.5.7.1 Prevention of Pancreatic Cancer

The finding of a PDAC-associated microbiome improves the possibility of its adoption as a noninvasive diagnostics tool for initial pancreatic cancer diagnosis utilizing faecal or oral samples. This testing has indicated potential in colorectal cancer; however, its application in PDAC is questionable (Villéger et al. 2018; Narayanan et al. 2014). Multiple investigations linked PDAC to the oral microbiota (Michaud et al. 2007; Fan et al. 2018). *Neisseria elongata* and *Streptococcus mitis* showed 96.4% sensitivity and 82.1% selectivity in recognizing pancreatic cancer comparison to healthy controls (Farrell et al. 2012). *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* increase pancreatic cancer risk (Fan et al. 2018). Prediagnostic blood samples from 405 patients with PDAC and 416 healthy controls were tested for antibodies to oral microbiota. Pancreatic cancer was shown to be two times more likely in patients with high levels of *Porphyromonas gingivalis* antibodies than in those with low levels (OR 2.14; 95% Cl 1.05, $p = 0.05$) (Michaud et al. 2013). Although the significance of the oral microbiota in PDAC carcinogenesis has not been established, it seems to be a promising diagnostic biomarker. Antibiotics taken orally can halt the spread of cancer, but studies have shown that transferring microbes or large amounts of faeces from PDAC-bearing mice to healthy mice has the opposite effect, hastening tumour development. These findings raise the possibility of using antibiotic therapy as part of chemotherapy for people with precarious pancreatic intraepithelial neoplasia (Pushalkar et al. 2018).

10.5.7.2 Enhance Anticancer Treatment Efficacy

Additionally, the microbiota may enhance the effects of cancer therapy. Anticancer efficacy of gemcitabine was restored in colon cancer mouse models after ciprofloxacin eradication of the intrapancreatic microorganism (Geller et al. 2017). Immunological mechanisms through microbiome have been identified to influence the efficacy of cyclophosphamide (Viaud et al. 2013a).

Cyclophosphamide damages the epithelium, causing a leaky gut and activating anti-tumour Th17 responses. Cyclophosphamide loses its anticancer activity in antibiotic-treated or gut-less mice (Viaud et al. 2013a). Zitvogel and colleagues evaluated anti-CTLA4 and anti-PD1 inhibitors (Zitvogel et al. 2018; Vétizou et al. 2015). When fed Bacteroides fragilis, microbe-free mice responded better to ICIs. In mice, cyclophosphamide develops resistance when the gut flora is eliminated with antibiotics, unlike gemcitabine (Viaud et al. 2013a). Oxaliplatin, a platinum drug often used in the FOLFIRINOX regimen, causes DNA damage and apoptosis by generating DNA adducts (Alcindor and Beauger 2011). Iida et al. found that the release of reactive oxygen species (ROS) from myeloid cells, an increase in the production of inflammatory cytokines, and tumour reduction in mice with the MC38 colorectal cancer and the B16 melanoma tumour was enhanced by a healthy microbiome (Iida et al. 2013). Antibiotics, on the other hand, reduced the effectiveness of oxaliplatin. Germ-free mice also decreased when there was no indigenous

flora (Iida et al. 2013). Anticancer treatment effects may be improved by altering an individual's microbiome, as demonstrated by these findings.

Pushalkar et al. found different bacterial compositions in human and animal PDAC compared to normal pancreas, suggesting digestive tract bacterial translocation into the intratumoral milieu (Pushalkar et al. 2018) Pushalkar et al. Their findings included the discovery that antibacterial ablation changed the tumour microenvironment, enhancing the activity of immune cells, as well as strengthening the patient's response to immunotherapy.

A novel approach to sensitizing PDAC tumours to ICI was thus suggested by Pushalkar et al. by altering the microbiome in the stomach and the tumour (Pushalkar et al. 2018). Due to PDAC's resistance to immunotherapy, it may be possible to make the microbiota more receptive to its effects.

10.5.7.3 Alleviate Side Effects of the Treatment

One of the more recognized side effects of ICIs is colitis. Patients with ICI-induced colitis have a different bacterial metagenomic profile, as discovered using metagenomic sequencing. Increased Bacteroidetes phylum bacteria were connected to a reduced risk of ICI-induced colitis in patients. The found microbial indicators may forecast the likelihood of ipilimumab-induced colitis in patients (Dubin et al. 2016). Mice treated with antibiotics had ICI-induced colitis alleviated by supplementation with bacteroides and burkholderiales, as well as FMT therapy (Vétizou et al. 2015; Wang et al. 2018).

FOLFIRINOX regimen includes irinotecan hydrochloride (CPT-11) as a topoisomerase-1 inhibitor. Active metabolite SN-38 is produced by liver carboxylesterases from CPT-11. High levels of SN38 can lead to diarrhoea because it damages the intestinal epithelial cells (Chen et al. 2013). Due to the fact that the inactive SN-38 G can be converted back to the active and poisonous form by gastrointestinal resident microbial β-glucuronidases (GUS), the adverse effects of CPT-11 may be due to these GUS (Ding et al. 2018; Panebianco et al. 2018).

Researchers found that CPT-11-based chemotherapy increased gut microbial dysbiosis by supporting potentially hazardous bacteria like Enterobacteriaceae and Clostridium spp. while lowering the prevalence of useful bacteria like Lactobacillus spp. and Bifidobacterium spp. (Lin et al. 2012). Enhancing the growth of good bacteria in the stomach and suppressing pathogens or opportunistic microorganisms can lessen the unwanted effects of CPT-11.

10.5.7.4 Microbiomes Act as Biomarkers in Pancreatic Cancer

The microbiome has the potential to serve as a biomarker for predicting health outcomes in the future. Metagenomic analysis was performed by Riquelme et al. on 68 resected PDAC tumour samples from two different cohorts: STS and LTS (Riquelme et al. 2019). Matching criteria included age, gender, and treatment stage for both groups. LTS patients had an average survival of 10.1 years, while STS patients had an average survival of 1.6 years. Patients in the LTS cohort of PDAC patients had a stronger anti-tumour response and immune system activation following FMT of their microbiome, compared to patients in the STS cohort (Riquelme

et al. 2019). Patients with PDAC may be able to use the microbiota as a predictive pointer in the future.

Thirty two pancreatic cancer patients, 32 autoimmune pancreatitis patients, and 32 healthy controls were studied by Zhou et al. PDAC patients' alterations in faecal bacteria and butyrate suggest a role for the gut microbiota in the disease's pathogenesis, and these changes could serve as biomarkers to help differentiate patients with pancreatic cancer from those with autoimmune pancreatitis and healthy controls. Though, to verify these findings, more research is required. There is some evidence that *Fusobacterium nucleatum* may function as a predictive biomarker for colorectal cancer although this has yet to be proven in the PDAC (Castellarin et al. 2012). *Fusobacterium nucleatum* was reported to enrich pancreatic tumour tissues in one investigation (Nejman et al. 2020). The microbiome has a diverse variety of prospective uses in the treatment of patients with PDAC as a whole. As detailed in this chapter, forthcoming studies should concentrate on efforts to clarify these possibilities for pancreatic cancer patients. Clinical studies on the microbiota in patients with pancreatic cancer are discussed next.

10.6 Clinical Studies Related to Gut Microbiome Modulation in Pancreatic Cancer

In order to successfully transition from the laboratory to the clinical sphere, numerous clinical trials examining the possible part of microbiome in the identification and management of pancreatic cancer have been conducted (Table 10.1). It will be easier to identify diagnostic biomarkers for pancreatic cancer if researchers explore the gut microbial makeup throughout the development of the disease (NCT03809247, NCT03840460). The surgical treatment of PDAC may benefit from a study now being conducted to examine the effects of gastrointestinal surgery on oral and faecal microbiomes. As an added advantage, researchers are investigating the impact of gut microbiota associated with PDAC on anti-MSLN CAR-T cell performance (Abdul Rahman et al. 2021; Sobocki et al. 2021). There is hope to gain a better knowledge of PDAC carcinogenesis and its possible involvement in treatment through these cooperative efforts.

10.7 Future Aspects

In order to better understand how microbiome modification affects pancreatic tumour development and treatment, more extensive studies are needed. Bacteria that can promote or impede pancreatic cancer development and treatment efficacy may provide a potential target or discover culprit pathways. Targeted microbial therapy for pancreatic cancer may enhance outcomes for patients. Bacteria, which make up the vast majority of the microbiota, are the subject of the most research into the host microbiome. However, host disease development has been shown to be affected by both the host viruses (virome) (Yu et al. 2021) and fungi (mycobiome)

Table 10.1 Clinical trials related to human microbiome in pancreatic cancer (Abdul Rahman et al. 2021; Sobocki et al. 2021; Yu et al. 2021)

Title of the study	Study type and objective	Country	Status
Oral microbiome and pancreatic cancer (NCT03302637)	A potential, observational, case-control study with 732 participants to relate oral and pancreatic microbiota to pancreatic cancer risk	United States	Completed
The microbiome of pancreatic cancer: "PANDEMIC" study (NCT04274972)	A prospective, observational, cohort study with 20 patients to describe the pancreatic microbiome of people with resectable PDAC who are getting a pancreaticoduodenectomy and figure out how the microbiome is linked to complications after surgery	Italy	Recruiting
A microbiome study of patients undergoing GI surgery for oesophageal, pancreatic, and colorectal cancers (MA-PPING) (NCT04189393)	In this study, 60 patients who had been diagnosed with pancreatic, oesophageal, and/or colorectal cancer at the time of their surgical patient journey were included	Netherlands	Active, not recruiting
Prognostic and predictive biomarker discovery in pancreatic ductal adenocarcinoma and pancreatic neuroendocrine tumours: results from a prospective translational tissue collection research (PaC-Man) (NCT03840460)	Specific intra-pancreatic colonizing microorganisms, molecular subtypes, and response and toxicity markers are all investigated in a prospective observational cohort research comprising 200 patients at varying stages of pancreatic lesions (from precancerous lesions to more advanced illness)	United Kingdom	Recruiting
Hypofractionated radiation therapy combined with the live biotherapeutic agent MRx0518 in resectable pancreatic cancer (NCT04193904)	15 patients with resectable pancreatic cancer to participate in an open-label interventional phase I research to assess the safety and preliminary efficacy of MRx0518 when given preoperative hypofractionated radiation	United States	Recruiting
Regulation of gut microbiota as a method for boosting the anti-tumour effects of CAR-T on pancreatic cancer (NCT04203459)	80 patients with pancreatic cancer enrolled in an observational cohort research to investigate how chimeric antigen receptor T cells enhance anticancer effects through regulating the gut flora	China	Recruiting

(continued)

Table 10.1 (continued)

Title of the study	Study type and objective	Country	Status
Microbial variety in pancreatic disorders (NCT03809247)	Potential biomarkers and pathogenic pathways that induce pancreatic illness are the subject of a retrospective observational study with 330 patients	China	Recruiting
Oral microbiome and pancreatic cancer (NCT03302637)	To further understand how oral and pancreatic microbiomes affect pancreatic cancer risk, researchers conducted a prospective, case-control study comprising 732 people	United States	Completed
Colonization of bile ducts and postoperative infectious complications of pancreaticoduodenectomies (NCT03525067)	The primary goal of this prospective observational cohort study, which included 46 participants, was to determine whether biliary colonization was associated with postoperative infectious complications, as well as to determine the overall morbidity and mortality for patients who had undergone pancreaticoduodenectomy	France	Completed

(Yu et al. 2021) as well. Recent research suggests fungi may contribute to pancreatic tumorigenesis. Aykut et al. found fungus in human pancreatic cancer tissues using a 28S rRNA probe (Yu et al. 2021). Mycobiome ablation with amphotericin B reduced oncogenic development and boosted gemcitabine's anticancer efficacy in the KC mouse model of PDAC. Fungi and viruses need more research to better understand their role in pancreatic carcinogenesis.

Numerous studies have indicated a relationship between pancreatic and other malignancies and the host's microorganisms and immune system; therefore, it is important to recognize the influence of definite microbial assemblages on anti-tumour immunity, tumour biology, and therapeutic response. Realizing individual microbial roles through their methods of action and metabolites may enable innovative pancreatic cancer treatment strategies. Pancreatic cancer and dietary manipulation of probiotics, prebiotics, and flora in the gut are understudied and require further attention as a noninvasive intervention to decrease PDAC risk or affect therapy response.

10.8 Conclusion

Pancreatic cancer is lethal and aggressive. Some pathways of pathogenicity have been found, including the link with Type 2 diabetes and pancreatitis. In spite of this, it is not apparent how to detect pancreatic cancer in its initial phases in order to prevent its development. Microbiota has a significant impact on pancreatic cancer growth and progression. Emerging evidence links a disrupted human microbiome to pancreatic cancer. Inflammation generated by microbes impacts oncogenic signalling, tumour cell metabolism, and the immune response to pancreatic tumours. Using microbiome profiling as a biomarker, we can detect those at elevated risk of pancreatic cancer. Evidence implicating the intestinal microbiome in the pathogenesis of pancreatic diseases bolsters the importance of the extensive gut–pancreas interaction, resulting in a heightened attention in microbiome characterization and engineering through the use of probiotics, prebiotics, synbiotics, postbiotics, or FMT. Although the gut microbiome is the well-studied, other microbiomes such as lung, skin, oral cavity microbiomes lead to pancreatic cancer progression and can boost or hinder cancer treatments. Extensive research have better comprehended the human microbiome and pancreatic cancer. New ways to addressing pancreatic cancer by modulating human microbiome may emerge. However, it is still not very clear if the human microbiome causes pancreatic cancer. Pancreatic cancer mechanisms mediated by the human microbiota have substantial clinical implications, leading to a unique and more specific strategy to modulate the human microbiome. The combination of immunotherapy and microbiome modification is a unique technique, but more study is needed to verify its efficacy in pancreatic cancer. Further preclinical and clinical studies are essential to know exactly how the human microbiota influences pancreatic cancer and in what way probiotics, prebiotics, synbiotics, and FMT can improve disease consequences. The ultimate goal of future research in this area is to implement individualized microbiome engineering in clinical practice.

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Uncharted Potentials of Synbiotics in Treatment of Cervical and Ovarian Cancer

11

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Abbreviations

ACS	American Cancer Society
AGS	Adenocarcinoma gastric cell line
AKAP4	A-kinase anchor protein 4 precursor
ARID1A	AT-Rich Interaction Domain 1A
AURKC	Aurora Kinase C
BCl2	B-cell lymphoma 2
BRAF	Serine/threonine-protein kinase B-Raf
BRCA1	Breast Cancer gene 1
BRCA2	Breast Cancer gene 2
CASP3	Cysteine-aspartic acid protease
CDK2	Cyclin-dependent kinase 2
CTAs	Cancer testis antigens
CTNNB1	Catenin Beta 1
FIGO	Fédération Internationale de Gynécologie et d'Obstétrique
HeLa	Henrietta Lacks
HPV	Human Papillomavirus
hrHPV	High-risk human papillomavirus
HT-29	Human Colorectal Adenocarcinoma Cell Line
IL-10	Interleukin 10
IL12	Interleukin 12
KRAS	Kirsten rat sarcoma viral oncogene homolog
MCF-7	Michigan Cancer Foundation-7

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miR-200b	MicroRNA-200b
miR-21	MicroRNA-21
MMP2	Matrix metalloproteinase-2
MMP9	Matrix metalloproteinase-9
NF-κB	Nuclear factor kappa B
OIP5	Opa interacting protein 5
PCOS	Polycystic ovarian syndrome
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha.
PPP2R1A	Serine/threonine-protein phosphatase 2A
PTEN	Phosphatase and Tensin
Th1	T helper type 1
TLR-4	Toll-like receptor 4
TNF-α	Tumor Necrosis Factor alpha
TP53	Tumor Protein P53
TSGA10	Testis Specific 10

11.1 Introduction

Cancer is the most prevalent cause of deaths worldwide (Momenimovahed et al. 2017) and also a major hindrance to achieve the desired life expectancy in most of the countries (Bray et al. 2018). Among the various cancers, ovarian cancer, a fatal gynecological malignancy, also known as high-grade serous ovarian cancer (HGSOC), is perhaps the most frequent malignancy among women across the world (Coburn et al. 2017). It is the fifth greatest cause of cancer mortality among women in Western Countries (Jemal et al. 2007).

The American Cancer Society (ACS) stated that in the United States alone, the newly diagnosed cases for ovarian cancer in the year 2022 are estimated to be about 19,880, with deaths of about 12,810 (Key Statistics 2022). ACS also stated that, during the lifetime of a woman, risk of being diagnosed with ovarian cancer is around 1 in 78 and possibility of death is 1 in 108. Nevertheless, ACS also reports that the recent cases for cervical cancer, for the year 2022, are estimated to be 14,100, with deaths of about 4280 women. There are basically two types of tumors in ovarian cancer namely, Type I and Type II (Kurman 2013). Type I tumors (30%) are low-grade tumors with mutations in the KRAS, BRAF, PTEN, CTNNB1, PIK3CA, PPP2R1A, and ARID1A genes (Shih and Kurman 2004; Wiegand et al. 2010). These tumors are indolent and have good prognosis. Whereas Type II tumors also referred as advanced-stage cancers, account for 70% of all the ovarian cancers. These are aggressive and are of high-grade exhibiting high mortality rate. Type II cancer features TP53, BRCA1, and BRCA2 gene mutations, and displays high level of genetic instability (Kurman 2013; Kurman and Shih 2011).

The risk of ovarian cancer increases with age and is at its peak among individuals at the age of 50–80 years (Roett and Evans 2009). Various factors that contribute to the progression of ovarian disease depend on the genetic, gynecologic, hormonal, and lifestyle conditions. The risk factors could also be related to epidemiological variability in various locations worldwide (Hunn and Rodriguez 2012). Commonly, ovarian cancer is observed in non-Hispanic white women, i.e., 12% of 100,000 women, followed by Hispanic women, i.e., 10.3% of 100,000 women. However, due to disparity in the access to diagnostics as well as treatment services, the mortality rate for ovarian cancer follows a distinct pattern with African women showing highest death rate worldwide (Torre et al. 2018). Other factors responsible for ovarian cancer include smoking and body mass index (Roett and Evans 2009). Nevertheless, the factors which decrease the number of ovulatory cycles including pregnancy, breastfeeding, and use of oral contraceptives are responsible for reduction in risk of ovarian cancer (Momenimovahed et al. 2019). Various hypotheses for etiology of ovarian cancer are still unclear. The role of hormones, immunology, inflammation, genetic alteration, and mutation theories are associated as potential causes according to various research carried out worldwide, but there is no clear evidence established.

With reference to cervical cancer, it is a gradually progressing disease and one of the major reasons of death in women worldwide. The lower section of the uterus, i.e., the cervix, is affected in patients suffering from cervical cancer. It is reported that annually there are over 530,000 new cases and 270,000 fatalities throughout the world, which reflects the severity of the disease (Small et al. 2017). Cervical cancer is associated to the long-term infection with greater risk of Human Papillomavirus (HPV) strains, type 16 and 18. Infections with HPV, if not treated at an appropriate phase, may result into cervical intraepithelial neoplasia (Kessler 2017). The risk factors directly linked to HPV infection include multiple sex partners, high-risk sexual relationships, and patient history of HPV-related vaginal dysplasia. Furthermore, women suffering from polycystic ovarian syndrome (PCOS) have metabolic abnormalities, which can also be responsible for increasing the risk of cervical cancer (Cohen et al. 2019).

Major risk factors responsible for the development of carcinogenesis at the cervix uteri are associated with smoking and HPV 16/18 virus (Zhang et al. 2020). Therefore, as a preventive measure, it is recommended to receive HPV vaccination. Across the world, methods like high-risk HPV genotyping, cell morphology screening to molecular testing, liquid-based cytology are few screening procedures carried out for early identification of the problem, which is also highly recommended by various medical associations. Big data technology is also playing a vital role in screening cervical cancer by incorporating Artificial Intelligence and integrating image recognition concepts (Hou et al. 2022; Wang et al. 2021).

Several therapies are available for cervical cancer management at the early stage, which include surgery, radiotherapy, neoadjuvant chemotherapy, to name a few. Nevertheless, other treatment options include concurrent chemoradiation in which cisplatin is administered alone or in combination with the other medications. This

therapy is the highly recommended therapy for patients suffering with locally advanced cervical cancer (Kumar et al. 2018).

11.2 Oncobiotic Biotransformation in Cervical and Ovarian Cancer

Oncobiosis is defined by the alterations in microbiome compartment across a wide range of neoplastic disorders. Several changes occur in microbiome compartment during ovarian and cervical cancer. Oncobiosis is seen in several compartments including cervicovaginal (Nené et al. 2019), ovarian and intratumoral compartment, upper and lower genital tract (Zhou et al. 2019), serum and intestines. *Lactobacilli* operate as gatekeepers against bacterial and/or viral infections in vaginal and cervical areas by (1) maintaining low pH; (2) sustaining healthy microbial homeostasis; (3) producing antimicrobial compounds namely, bacteriocins and hydrogen peroxide, that are capable of overpowering the growth of undesired microbes; and (4) modulating local immune system (Valenti et al. 2018; Łaniewski et al. 2020). *Lactobacilli* is generally responsible for the prevention of ovarian cancer, owing to the above-mentioned benefits offered by the bacteria (Xu et al. 2020). Whereas literature cites studies wherein *Lactobacillus*-deficient vaginal communities were found to be common in ovarian cancer patients than in controls. An inflammatory potential is linked with the colonization of Gram-negative bacteria, which is found to be responsible for the initiation phases that lead to carcinogenesis. This may be attributed to the increase in oxidative stress, damage to the DNA or by the accumulation of mutations (Sipos et al. 2021).

Lactobacillus spp.-deficient communities are common among BRCA (1/2) mutation carriers that signify the importance of oncobiosis in amplifying the effects of genetic mutations (Nené et al. 2019). In the tumor tissue, the ratio of *Proteobacteria-to-Firmicutes* increases as the abundance of *Proteobacteria* increases (Wang et al. 2020). The number of *Fusobacteria* is also found to be greater in tumor cells than in healthy non-transformed tissues. As *Proteobacteria* and *Fusobacteria* both are Gram negative, the microbiome becomes more immunogenic and there is abundance of Gram-negative bacteria in oncobiotic peritoneal membrane (Miao et al. 2020). On the contrary, the gut oncobiome is dominated with Gram-positive bacteria with the number of bacteroides decreasing, while the number of Firmicutes, Actinobacteria, and Proteobacteria and increasing (Mori et al. 2019).

The female vaginal tract is protected from infectious pathogens through a number of defensive systems which include the presence of mucosal epithelial barrier, secretion of mucus, lactic acid production, and immunological responses. The vaginal mucosa is a barrier which protects the infections as a result of interaction between epithelial cells, the immune system as well as colonization of favorable microorganisms. Audirac-Chalifour et al. investigated the cervical microbiome and cytokine profiles in patients suffering from cervical cancer at various stages. They postulated that the microbiome composition changes from *L. crispatus* to *L. iners* following hrHPV infection of the cervical epithelium. When an infection advances

to a squamous intraepithelial lesion, *Sneathia* and *Fusobacterium spp.* show a rise in microbial diversity. *Fusobacterium necrophorum* was also found in cervical cancer, adding to the microbiome's variety. In this paradigm, HPV infection causes an immunosuppressive microenvironment (through IL-10 production and macrophage type 2 activation) that is exacerbated by microbiota-derived TGF-1, resulting in a positive feedback loop between microbiota and cytokine profile (Audirac-Chalifour et al. 2016). Lactic acid inhibits the growth of numerous anaerobic agents linked to sexually transmitted illnesses, which may aid in the evolution of cervical lesions when hrHPV persistence is present (Robial et al. 2017). *C. trachomatis* infection appears to enhance the chance of hrHPV infection in cervical cancer through an inflammatory response that increases ROS generation and free radical production (Zhu et al. 2016). Di Pietro et al. focused on the different types of cervical microbiomes related with *C. trachomatis* and HPV infection. Women with both the infections had more bacterial variety, which was mostly due to the presence of anaerobes such as *G. vaginalis*, *A. vaginae*, and lower quantities of *Lactobacillus* indicating a link between dysbiosis and infection. Healthy women, on the other hand, had a *Lactobacillus* dominance, with anaerobic bacteria accounting for just 2% of the cervical flora. Women infected alone with *C. trachomatis* showed a varied cervical flora, but low levels of *L. iners* as compared to healthy women (Di Pietro et al. 2018).

11.3 Chemotherapeutics in Cervical and Ovarian Cancer

11.3.1 Cervical Cancer

Depending on various factors like stage and type of cancer, side effects, patient's overall health and treatment choice, therapy to combat cancer can vary from radiation, surgery or via medications through chemotherapy, targeted therapy, and immunotherapy, (Fig. 11.1) to name few.

Chemotherapy is indeed an essential conventional treatment option for cervical cancer. As adjuvant therapy it is often given at post-surgery, when the tumor

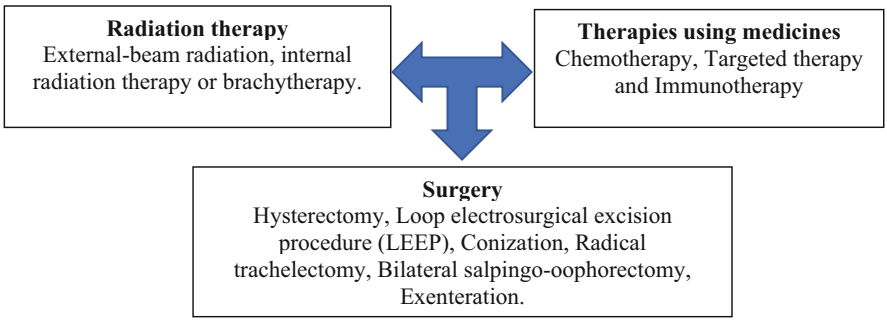

Fig. 11.1 Various types of cancer therapies

characteristics enhances the likelihood of recurrence, in conjunction with radiation or as alone treatment. Cisplatin is a platinum-based chemotherapeutic agent that has been used in the treatment of cervical cancer since last three decades (Tewari and Monk 2005). The standard first line agent used in chemotherapy is based on the combination of paclitaxel and cisplatin combination. This combination has shown superiority in terms of progression-free survival when compared to cisplatin monotherapy (Moore et al. 2004). However, in 30–40% of cases there is a lack of response with this combination. The other combination therapies with cisplatin include topotecan, bleomycin, and 5-flourouracil (Tewari and Monk 2005). In the selection of treatment regimen for recurrent cervical cancer, there was no treatment which outperformed in terms of recurrence both inside and outside the pelvic cavity. These results highlight the complexities and obstacles of creating curative treatment for individuals suffering from recurrence of cervical cancer. A novel treatment is required which might halt the cycle of recurring cervical cancer. The tailored therapy of surgery as well as radiation was found to be more beneficial than the other combinations (Wang et al. 2011).

11.3.2 Ovarian cancer

According to Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) system, ovarian cancer is staged into four stages, which takes into account the degree of the tissue involvement, the status of lymph node, and the severity of metastasis. The malignancies in stage 1 and 2 are restricted to the pelvic cavity and are referred to as early-stage cancer, whereas stage 3 and 4 are beyond the pelvic cavity and are referred to as advanced stage cancer (Prat et al. 2015). The conventional treatment for advanced ovarian cancer is initial cytoreductive surgery which is followed by platinum-based chemotherapy. The first-line treatment with paclitaxel and carboplatin improves clinical response although recurrence develops in 25% of patients with early-stage cancer. Recurrence also occurred in more than 80% patients with the advanced disease (Agarwal and Kaye 2003). Majority of patients suffering from advanced stage disease had recurrence within 2 years of combination therapy. Drug resistance is one of the main reasons of chemotherapy failure in advanced ovarian cancer. The intrinsic chemoresistance is produced by cancer cells by several biological modifications including decreased drug absorption, suppression of apoptosis, etc. (Rubin et al. 1999). The main issue here includes identification of individuals who are predisposed to chemo-resistance as current diagnostic tools are unable to aid doctors in making an educated choice to change treatment course prior to chemotherapy.

11.4 Challenges Faced During Cancer Therapy

The prime issue encountered during chemotherapy is that the therapy works by damaging both the healthy cells and cancerous cells. Hence, the adverse effects of chemotherapy include nausea, vomiting, low in mental focus, nerve pain, ortho pains, hair loss, to name few. At several instances, recurrence of cancerous cells may be observed, as they compromise the immune system. The human microbiome plays a vital role in pathophysiology of cancer; use of synbiotics may be beneficial in manipulating the microbiota, enhancing the potential outcome of therapeutic agents and reducing the undesirable effects of therapy in cancer patients. The mechanism of synbiotics and their clinical benefits in cervical and ovarian cancer patients are at the infancy stage; nonetheless, there are studies showing the onco-suppressive effects of synbiotics in other cancers, which are ascribed to immunomodulation, metabolism, etc. Synbiotics also act by maintaining the intestinal barrier functions and preventing the host cell proliferation (Scott et al. 2018).

11.5 Probiotics, Prebiotics, and Synbiotics

The term "biotic" originates from a Greek word meaning "life" (Pandey et al. 2015; Ozen and Dinleyici 2015). Probiotics are living bacteria that possess numerous positive effects on their host when consumed in sufficient quantities (Liang et al. 2019). Some of the most effective probiotics comprise of microbes belonging to *Lactobacilli*, *Bifidobacteria, Lactococci*, *Streptococci*, and *Enterococci* species, which typically produce lactic acid and could be either fermentive, obligatory, or facultative anaerobes (Ozyurt, and O¨tles, S. 2014). Non-pathogenic strains of *Escherichia coli*, some bacilli and yeast strains, also fulfil the definition of probiotics. On the other hand, prebiotics consist of a substrate that are selectively used by host microbes to provide health benefits (Swanson et al. 2020). Prebiotics generally include bifidogenic, non-digestible carbohydrates such as galacto-oligosaccharides, fructo-oligosaccharides, lactulose, inulin, to name a few (Pandey et al. 2015). According to the International Scientific Association for Probiotics and Prebiotics (ISAPP), the term "synbiotic" expresses "a regulated mix of live microbes and substrate(s) selectively utilized by host microorganisms that confer a health benefit on the host," wherein the "host" microbes could be both autochthonous (resident in the host) and allochthonous (externally applied, such as probiotics) (Swanson et al. 2020).

As reiterated earlier, vaginal dysbiosis along with unhealthy diet or sedentary lifestyle have been responsible for the progression of cervical cancer, while gut microbiome is found to be responsible for the development of ovarian cancer (AlHilli and Bae-Jump 2020). Probiotics lower the risk of cancer by controlling the microbiome and decreasing the inflammation. Probiotics have received a lot of interest recently due to their capacity to affect the cancer cell growth and death. The maximum utilization of the features of probiotics might be a new choice to the intrusive treatments including radiation and chemotherapy. Nami et al. isolated

L. plantarum 5BL strain from vaginal secretions of healthy, young Iranian women and assessed its cytotoxic property against different cell lines (i.e., HeLa, MCF-7, AGS, and HT-29). The study results showed that the isolated strains exhibited probiotic properties including low pH, tolerance against high bile salt levels, antibiotic susceptibility and antibacterial activity against few pathogens (Nami et al. 2014). Nouri and co-workers studied the anti-proliferative effect of *L. rhamnosus* and *L. crispatus* on HeLa cell growth, wherein they reported the downregulation of expression of CASP3 gene, MMP2 and MMP9, and upregulation of expression of TIMP-1 and TIMP-2 genes, confirming an inhibitory effect on metastasis (Nouri et al. 2016). Studies carried out by Wang et al. have shown that *L. crispatus*, *L. jensenii*, and *L. gasseri* were able to prevent the proliferation of CaSki cells (i.e., cervical cancer cells). This activity can be attributed to downregulating the expression of cyclin A and CDK2, while upregulating the expression of p21 genes. This resulted in a modification in the cell cycle, which caused cell arrest in S phase and decreased the number of CaSki cells in G2/M phase (Wang et al. 2018). On similar lines, Rajoka and coworkers isolated several *Lactobacilli* from healthy human breast milk, among which, *L. casei* (SR1 and SR2) and *L. paracasei* (SR4) strains demonstrated greater resistance against several antibiotics, high bile salt content, and acidic pH. Additionally, they showed significant antioxidant activity by quenching the free radicals, and remarkable anticancer activity against cervix cancer (HeLa) cell lines. The antitumor activity of the strains was attributed to upregulating the expression of BAX, BAD, caspase-3, -8, and -9 genes and downregulating the expression of BCl2 genes (Rajoka et al. 2018). Nouri et al. investigated the effect of *L. rhamnosus* GG and *L. crispatus* SJ-3C-US on expression of four cancer-testis antigens (CTAs), i.e., TSGA10, AURKC, OIP5, and AKAP4, in HeLa cell line, wherein the *Lactobacilli* downregulated the expression of CTAs. According to their observation, the epigenetic modulatory mechanisms could be responsible for the anticancer property (Nouri et al. 2018). Yousefi et al. in their review have discussed the immunomodulatory properties of probiotics and have mentioned that probiotics reduce inflammation by suppressing different signalling pathways including nuclear factor (NF)-κB pathway (Yousefi et al. 2019). Isolated strains of *L. plantarum* (NK3) and *B. longum* (NK49) by Kim et al. have shown to inhibit NF-κB activation and TNF-α expression in the vagina and uterus of a mouse (Kim et al. 2019). Dwi Ningtiyas et al. studies revealed that the intra-and extracellular extracts of *L. plantarum* IIA-1A5 and *L. acidophilus* IIA-2B4 were capable of inhibiting cervical cancer HeLa cells. Among the two sources, the intracellular extracts exhibited higher inhibitory effect when compared to the extracellular extracts, owing to the presence of proteins and other compounds (Dwi Ningtiyas et al. 2021).

Negi and co-researchers developed cisplatin-cum-*L. rhamnosus* loaded pessaries, prepared by melt mold method, to provide benefits over the conventional drug therapy. Here the researchers, with the use of their dual-loaded pessary intended to circumvent the unwanted effects of chemotherapy and enhance the therapeutic activity (Negi et al. 2020). On the contrary, studies carried out by Kim et al. showed that *L. casei* extract had no synergistic effect after concomitant administration of one

or more chemotherapeutic drugs, namely, 5-fluorouracil, cisplatin, doxorubicin, and paclitaxel. Instead, their studies revealed that *L. casei*-induced S-phase cell cycle arrest in cervical cancer cells when administered with the anti-cancer drugs, other than 5-fluorouracil and concomitant administration of cisplatin with *L. casei* exhibited inhibitory effect on apoptosis. However, *L. casei* showed no significant effect on the growth rate of human cervical cell lines, CaSki and HeLa (Kim et al. 2015). Rahbar Saadat and co-workers studied the effect of vaginal isolated probiotic strain, *Lactococcus lactis* on CAOV-4 cells. The probiotic downregulated Toll-like receptor 4 (TLR-4), miR-21, and miR-200b expression levels, the factors responsible for the initiation and progression of ovarian cancer. The results were also partly validated using an in silico model (Rahbar Saadat et al. 2020).

While the majority of the research work emphasizes on the effect of probiotics on cervical cancer cells, studies from other cancer types suggest that synbiotics will surely have a great impact on the management and treatment of ovarian and cervical cancer. Nevertheless, research work carried out in this area is limited. This suggests more research to develop synbiotics with the mechanistic explanation for their efficiency.

11.6 Role of Synbiotics in the Treatment and Diagnosis of Cervical Cancer

Various treatment options for cervical cancer include chemotherapy, radiation, and surgery. However, use of these treatments is often impeded by low effectiveness, increased toxicity, and adverse effects (Datta et al. 2015). Current epidemiological and experimental research demonstrated a strong relationship between increased probiotic use and decreased cancer development. Research findings suggest that the anticancer properties of probiotics can be identified through various mechanisms including immunomodulation via enhancing or suppressing the molecular signalling pathways, microbiota pattern modification, and induction of apoptosis in cancerous cells (Śliżewska et al. 2020). *Bifidobacterium* is the significant component of gut microbiota that has been shown to interact and influence the immune system through innate and adaptive immunological pathways (Belkaid and Hand 2014). Abdolalipour and co-researchers examined and compared the effectiveness of oral versus intravenous probiotic delivery of *B. bifidum* in tumor bearing mice by activation of antitumor immunity. The findings clearly showed that intravenous or oral treatment of *B. bifidum* may inhibit tumor development by modulating the immune system via promotion of IFN-α and IL12 release in spleen cell culture, as well as Th1 response (Abdolalipour et al. 2020). Li and co-workers studied the anticancer effects and probable mechanisms of *Lactobacillus*. Cell Counting Kit-8 tests were used in order to identify appropriate dosages for studying the inhibitory impact of *Lactobacilli* on HeLa cell lines and U14 cell migratory abilities in vitro. The findings suggest that live *Lactobacilli* have the capacity to block cervical cancer cell migration with the probable pharmacological mechanism being closely tied to E-cadherin overexpression (Li et al. 2017). Cha et al. investigated the antiviral effect

of *B. adolescentis* SPM1005-A in the SiHa cervical cancer cell line expressing HPV type 16. The researchers found that *B. adolescentis* SPM1005-A possesses antiviral activity by suppressing the expression of the oncogenes E6 and E7. The findings show that *B. adolescentis* SPM1005-A might have implications in the prevention of HPV-associated cervical cancer (Cha et al. 2012). Sungur et al. discovered that *L. gasseri* (G10 and H15) strains isolated from human vagina suppress the growth of HeLa cells. The lyophilized exopolysaccharides from different strains of *L. gasseri* triggered apoptosis in HeLa cells in a strain-dependent manner. They inhibited cell proliferation and regulated their immune response. The strain G10's capacity to trigger apoptosis was linked to an increase in Bax and Caspase 3. *L. gasseri* strains inhibited inflammation in HeLa cells by lowering TNF-α production and enhancing IL-10 production (Sungur et al. 2017).

Cervical cytological diagnosis is generally affected due to vaginal infections caused by the accumulation of several microbes, white blood cells, and so on. In order to improve the cervical cytological diagnostic and make it more dependable, Perišić et al. showed that the use of anti-infectives along with probiotics, *L. rhamnosus* GR-1 and *L. reuteri* RC-14 effectively reduced the false-negative and false-positive results of cervical malignancies (Perišić et al. 2011). On the other hand, Ou et al. investigated the use of U-relax® (U-relax, Tri-factor Biotech Inc., Taiwan), an oral probiotic loaded with *L. rhamnosus* GR-1 and *L. reuteri* RC-14, to improve the reliability of cervical cytology. The outcome of their study revealed that there was no significant influence on genital HR-HPV clearance; however, they were able to minimize the anomalous rates of cervical smears (Ou et al. 2019).

11.7 Future Perspective and Conclusion

The chapter describes the effects of pro-, pre-, and synbiotics in cervical cancer and ovarian cancer. Probiotics do eliminate various side effects which are caused due to radiation, chemotherapy, and surgery. The outcomes which are favored by the probiotic treatments are only limited to the preclinical settings. Various studies including long-term studies for the method standardization, toxicity studies should be carried out for the probiotic strains on a larger scale, so the results available would be evident to prove the efficacy of probiotics. The effective therapy for cervical and ovarian cancer is directly linked with the dose of the probiotics, the bacterial or fungal strain used for the preparation and the time of exposure. As mentioned, the results provided by various probiotics in cervical cancer is limited to experimental settings only. So, regulatory body should establish rules related to the probiotics use in treatment of cancer and the long-term studies should be initiated. There is a need of clinical trials in future to establish the clinical benefits of synbiotics in cervical and ovarian cancer.

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Synbiotics in Buccal Cancer

12

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12.1 Introduction

According to the recent report of the American Cancer Society 54,000 cases of oral cancers are reported in a year (The American Cancer Society n.d.). Oral cancers emerge from the oral cavity which includes tongue, lips, gums, oral mucosa, floor of mouth, hard palate, maxilla, mandible and the pharyngeal cancers are the most commonly seen. Among all oral cancers 90% cases are oral squamous cell carcinoma (OSCC) (Zhang et al. 2020). The factors which induce OSCC are bad oral hygiene conditions, chewing tobacco, improper dietary conditions which include heavy consumption of alcohol. Annually, 6.4 million deaths are reported due to consumption of tobacco. Smoking is the prime factor in the development of oral cancer, certain flavours added by tobacco company in cigarettes are responsible for induction of carcinogenesis. However, it has been found that flavours like 4-(nitrosomethylamino)-1-(3-pyridyl)-1 butanone (NNK) and N′-nitrosonornicotine (NNN) are the two most hazardous flavours which induce tumour in oral cavity. NNK and NNN cause tumour in oral cavity by adducting DNA of keratinocyte stem cells which is responsible for mutations in DNA replication (Kakabadze et al. 2020).

Apart from tobacco, it has been found that certain bacteria present in oral cavity induce OSCC. Bacteria like *Porphyromonas gingivalis* responsible for tumour generation in squamous cells present in oral cavity. Also, *Pseudomonas aeruginosa (P. aeruginosa)* due to its metabolite properties acts as carcinogenesis in OSCC. *P. aeruginosa* elevates the concentration of nitric oxide (NO) which is responsible for apoptosis, angiogenesis, invasion and metastasis in cell cycle. Bacteria like

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Fusobacterium nucleatum and *Fusobacterium periodonticum* are also responsible for the development of OSCC (Meurman 2010).

Recent treatments for OSCC including chemotherapy, radiotherapy and surgery have high risk of complications and in most of the cases bacteria improve resistance. Treatment of OSCC by radiation therapy brings complications like caries by radiation, mucositis induced by bacteria and fungi, subcutaneous fibrosis, osteoradionecrosis and severe pain. Similarly, severe complications such as bone marrow suppression, renal, pulmonary ototoxicity and haematologic toxicity occur during chemotherapy (Huang and O'Sullivan 2013). Thus, an alternative solution required for the treatment of OSCC with less side effects and high therapeutic values.

Oral microbiota plays an important role in oral cavity by maintaining the immunity and nutritional status. Combination of probiotics and prebiotics (synbiotics) has effective anticancer actions against cancer inducing microorganisms. Also, synbiotics are potent in modification of chemo/radiation therapy. Furthermore, synbiotics enhance the effects of chemotherapeutic drugs and reduce the associated side effects (Devine and Marsh 2009). Thus, synbiotics are effective in inhibiting factors responsible for OSCC.

Probiotics are living microorganisms which have health benefits on the host body when administered in adequate amount. Probiotics are available in fermented food with nutritional and therapeutic value. Prebiotics are non-viable food components that have benefits on the health of host associated with modulation of the microbiota. Prebiotics are mainly found in inulin, dietary fibres, oligofructose, galactooligosaccharides, etc. Synbiotics are combination of probiotics and prebiotics, i.e. prebiotics enhance the activity of probiotics. Synbiotics produce a synergistic effect that helps in improving the activity of microbiota (Raman et al. 2013).

Probiotics show anticancer effects through mutagen binding, degradation and inhibition of mutagenesis. Potential probiotic strains attach to the mutagens through sugar and peptidoglycans to induce anti-mutagenic and anti-carcinogenic activity. Cell wall of probiotic strain *Streptococcus cremoris* (*S. cremoris*) Z-25 binds to carcinogenic heterocyclic amine 3-amino-1,4 -dimethyl-5H-pyrido-[4,3-b] indole (Trp-P-1) and 3-amino-1-methyl-5H-pyrido [4,3-b] indole (Trp-P-2). The binding effect of probiotics depends upon chemical nature, pH and the range of polysaccharide present on the cell wall receptor site. Similarly, probiotics prevent the benign procarcinogens transformation to toxic and highly reactive carcinogens. Probiotics in addition with dietary supplements assist in detoxification and metabolism of procarcinogens and carcinogens into less toxic metabolites, which prevents the formation of tumour (Zhang and Ohta 1991). Lactobacillus strains obtained from different dairy products have enhanced antigenotoxicity (>80%) against 4-nitroquinoline 1-oxide (4-NQO) (Cenci et al. 2002). Probiotic strains also perform anticarcinogen effect by enhancing host's innate immunity by secretion of anti-inflammatory molecules. It has been found that Methylcholanthrene-induced tumour mice, when orally administered with *Lactobacillus casei* strain Shirota (LcS) showed increased innate host immunity by activating the splenic NK cell leads to a decrease in tumour development (Yasutake et al. 1999).

Prebiotics possess anti-carcinogenic effect by proliferating the important components of oral microbiota indirectly. Another important aspect of prebiotics is producing short chain fatty acids (SCFA) and lactic acids which may play a key role in maintaining oral health, morphology and function. SCFAs are generally formed by acetic acids, propionic acids and butyric acids in a molar ratio of 60:20:20. SCFAs are involved in cell differentiation, termination of cell proliferation and induce apoptosis to eliminate the DNA-damaged cells which lead to malignancy (Jan et al. 2002). Lactate may improve the activity of oral microbiota by enhancing their immunity and surface adsorption mechanism (Scott et al. 2013). Furthermore, prebiotics may helpful in enhancement of micronutrient adsorption in oral cavity. Another possible anticarcinogen mechanism of prebiotics is modification of xenobiotic metabolising enzymes. Xenobiotic metabolising enzymes are indications of carcinogens. They are mainly categorised into phase I (cytochrome-b5, cytochrome-b5 reductase, cytochrome P450, cytochrome P450 reductase, etc.) and phase II (glutathione S-transferase, Uridine 5′-diphospho-glucuronosyltransferase and DT-diaphorase) enzymes reduce the activation of procarcinogens into active carcinogens and its termination from the body (Johnson et al. 2012).

Another interesting molecule developed by the researchers are the postbiotics. These postbiotics are the non-viable parts of the probiotics also known as ghost probiotics. These are dead cells, cell fractions or metabolites, enzymes of live probiotics which are obtained through fermentation process and perform several benefits to the host body after administered in sufficient amount. To overcome some issues generated with live probiotics, these microbial derived biomolecules known as postbiotics are developed. Postbiotics have several benefits like less side effects, high self-life, nontoxic, resistance to hydrolysis and stable in the gastric environment and have been used as adjuvant therapy in the colorectal cancer cases (Rad et al. 2021). Thus, postbiotics could be effective treatment for OSCC.

Synbiotics that may show anti-carcinogenic effect in oral cancer by a single or combination of probiotics strains are chosen based on the specific host interests, and prebiotics are selected independently to enhance the survival, growth and activity of oral microbiota. An ideal synbiotics supplement should have a single or multi-strain probiotic and an appropriate mixture of prebiotics, which favour the production of beneficial oral microbiomes and reduction of cancerous cells in oral cavity (Raman et al. 2013).

12.2 Probiotics

The probiotic concept was first purposed by Nobel laureate Metchnikoff in 1907, which means “for life”. Then Fuller, in 1989 defined probiotic as “A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance”. However, World Health Organization (WHO) and Food and Agriculture Organization of United States (FAO) defined probiotics as “Live microorganisms which when administered inadequate amounts confer a health benefit on the host” (Zendeboodi et al. 2020). The most predominantly available

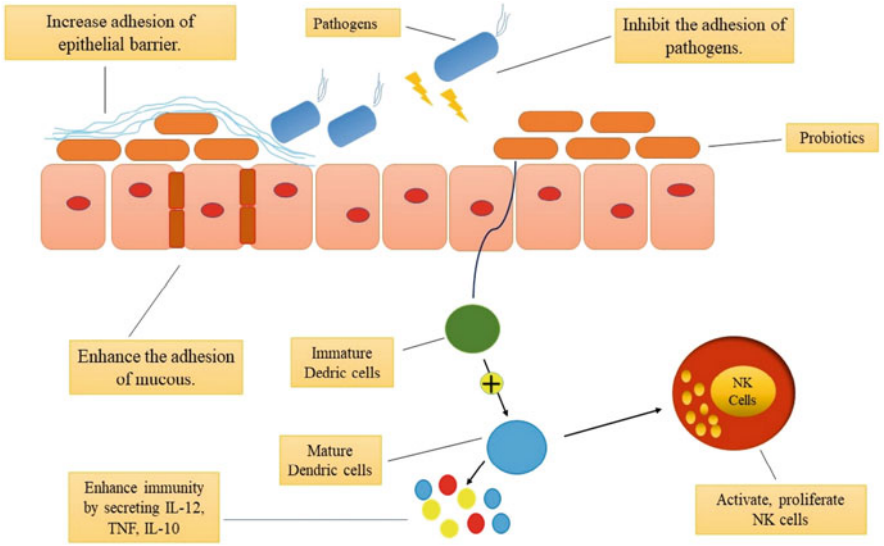

Fig. 12.1 General apoptosis mechanisms of probiotics against cancer cells

probiotic strains belong to genera *Lactobacillus* and *Bifidobacterium*. These probiotic strains perform various mechanisms such as interaction with nutrients, growth factors, and pH of residual bacteria. Probiotics also have effective against reduction of the toxin products of antimicrobials and anti-oxidants. Also, probiotics increase the immunity of residual bacteria by the activating t-lymphocytes replication. Furthermore, probiotics improve the efficiency of mucosal barrier, induce production of short chain fatty acids, metabolise bile acids and synthesise vitamins. Probiotics are further differentiated into two groups—live probiotics and dead probiotics. Generally, probiotics are living bacterial strains, but some dead bacterial strains have enhanced anti-inflammatory effects in host body. The anti-tumour effects of dead probiotics in higher doses are more as compared to the live probiotic strains. Dead probiotic strains showed anti-tumour activity by increasing secretion of IgA and stimulating host immune system (Legesse Bedada et al. 2020). The anti-carcinogenic mechanism of probiotics against cancer cells are illustrated in Fig. 12.1 (Table 12.1).

Probiotics possess anti-proliferative actions against squamous cell carcinoma of the oral cavity. Aghazadeh et al. investigated that probiotic strain *Acetobacter syzygii* (*A. syzygii*) has cytotoxicity against human oral cancer cell line KB and normal epithelial cell line KDR. It has been shown that *A. syzygii* showed 75.7% of apoptosis against KB cancer cell lines equivalent to cisplatin and only 9.36% of apoptosis against KDR normal cells. Experimental evidences showed that *A. syzygii* showed anticancer activity by bacterial secretory products (Aghazadeh et al. 2017). Also, it has been reported that *Lactobacillus acidophilus* (PTCC 1643) strain has useful effects on prevention of oral diseases. Further, Wang et al. investigated that probiotic strain *Lactobacillus salivarius (L. salivarius)* Ren has the potential to

Table 12.1 Probiotics in oral cancer

Sl. No.	Probiotics strains	Oral cancer types	Outcomes	References
1	*Lactobacillus salivarius* REN	Oral carcinogenesis [TCA-8113 cell line]	Decrease the cell line proliferation in 3×10^9 cfu/mL dose of the probiotic strain	Wang et al. (2016)
2	*Lactobacillus plantarum*	Human oral KB cancer cell line	Decrease the harmful tumour development by upregulating and downregulating the PTEN and MAPK pathways	Asoudeh-Fard et al. (2017)
3	*Acetobacter syzygii*	Human oral KB cancer cell line	The strain showed significant cytotoxicity against oral KB cell line	Aghazadeh et al. (2017)
4	*Lactobacillus salivarius* REN	4-nitroquioline 1-oxide (4NQO)-induced oral carcinogenesis	Significant effect in inhibiting oral carcinoma	Zhang et al. (2013)
5	*Lactobacillus rhamnosus* GG strain	HSC-3 human oral squamous carcinoma cells	This strain can enhance the anticancer activity of HSC-3, particularly with Geniposide	Cheng et al. (2017)
6	*Lactobacillus* sp. A-2 metabolites 1 and 2 (LM1 and LM2)	Tongue squamous carcinoma [CAL-27] cells	3–48 mg/mL of LM1 or LM2 can inhibit cell growth in dose-dependent manner	Zhang et al. (2014)

inhibit the apoptosis, cell proliferation, COX-2 mRNA levels and protein expression in oral cancer cell line (TCA-8113). Experimental evidence showed that *L. salivarius* Ren reduces the proliferation of cell lines at higher dose 3×10^9 cfu/mL. Treatment with higher dose of *L. salivarius* Ren (3×10^9 cfu/mL) showed 10.32% of early apoptosis and 28.15% of late apoptosis. Also, it has been seen that *L. salivarius* Ren reduces the formation of COX-2 mRNA formation up to nine-fold after 12 h of treatment. Thus, *L. salivarius* Ren has preventive effects against oral malignancy (Wang et al. 2016). In another study, Asoudeh-Fard et al. reported that *Lactobacillus plantarum (L. plantarum)* strain present in human oral cavity possessed reduction of oral cancer KB cells. *L. plantarum* showed potential signal transduction process by upregulating and downregulating PTEN (phosphate and tensin homologue) and MAPK (mitogen-activated protein kinase) gene expression. It has been found that after 24 h treatment 50.24% apoptosis occurred in the conditioned media. Experimental findings revealed that *L. plantarum* strain has potential effects in the treatment of oral cancer (Asoudeh-Fard et al. 2017).

Probiotics are also beneficial in avoidance of harmful effects of chemotherapy which leads to oral mucositis during the treatment of head and neck squamous cell carcinoma treatment. Sharma and co-workers investigated that administration of *Lactobacillus brevis (L. brevis)* CD2 lozenges reduce the seriousness of oral mucositis and side effects of chemo-radiotherapy. It has been shown that higher proportion of population (28%) remained free from mucositis as compared to the

placebo (7%) when treated with *L. brevis* CD2. This finding revealed that *L. brevis* CD2 lozenges are safe and effective in comparison to chemo-radiotherapy in the treatment of oral mucositis (Sharma et al. 2012). In another study, Feng et al. found that oral administration of probiotic strains reduces the chances of chemo-radiotherapy-induced diarrhoea and oral mucositis. The meta-analysis data revealed that the effectiveness of probiotic strains in prevention of harmful effects is caused by chemo-radiotherapy (Feng et al. 2022).

Probiotics are emerged as an alternative biotherapeutics and are effective in the treatment of different forms of cancer. Particularly, probiotics are effective in the treatment of oral cancer in the form of dietary supplements, nutraceuticals and nano-formulations. For oral administration of probiotics, the selection of beneficial strains of bacteria is important for higher therapeutic values. The modes of administration of probiotics should be enhanced to provide sufficient retention and exposure times in oral cavity which will enhance the penetration of mucosal biofilms and interactions with the microbial metabolism. Further studies are required to enhance the effectiveness of probiotics strains in the treatment of oral carcinoma.

12.3 Prebiotics

Prebiotics are indigestible dietary supplements which are used to develop the effectiveness of probiotics in the treatment of oral diseases. Prebiotics stimulate the growth and activity of probiotics and also reduce the effects of harmful bacterial strains. Also, prebiotics can alter the luminal and systemic characteristics of host immune system. Prebiotics are mainly dietary fibres and also available in the form of nutraceuticals. Natural source of prebiotics are bananas, asparagus, garlic, tomato, onion, etc. In general, prebiotics proliferate the activity of probiotics by indirectly. But in some cases, prebiotics also show direct effect on the host by stimulating expression of IL-10 and interferon γ, increasing secretion of IgA, modifying the inflammatory responses towards pathogens and also stabilising the mucosal barrier (Raman et al. 2013).

Major prebiotics such as curcumin, tea polyphenols and oligosaccharides have potential activity in the treatment of oral cancer. Zlotogorski et al. found that dietary compound curcumin has anti-neoplastic properties. The in vivo and in vitro studies of curcumin have showed the anti-neoplastic effects against OSCC in single as well as combinational methods. Curcumin downregulates the nuclear factor-kB (NF-kB) stimulation which is responsible for tumour growth. It has been found that curcumin shows anti-tumour effects by modification of the essential components present inside the tumour. Furthermore, curcumin enhances the cytotoxicity effects of CD8(+) T cells towards tumour generations, which result in tumour suppression (Zlotogorski et al. 2013a) In another study, Zlotogorski et al. investigated that nutraceuticals like green tea extracts [(-)-epigallocatechin-3-gallate (EGCG)] and polyphenolic resveratrol also have anti-tumour activity in oral cancer. The anti-tumour activity of EGCG and resveratrol are similar as curcumin (Zlotogorski et al. 2013b). The main challenges in administration of these compounds are poor bioavailability in oral

Table 12.2 Prebiotics in oral cancer

Sl. No.	Prebiotics	Outcomes	References
1	Oligosaccharides	Increase the growth and activity of *Bifidobacterium* and *lactobacillus*	Gibson and McCartney (2005)
2	Curcumin	Show anti-tumour activity by modifying the microenvironment of tumour	Zlotogorski et al. (2013a)
3	Polyphenols	Tea polyphenols are effective in the treatment of oral cancers	Ding et al. (2013)

surface. To overcome these challenges, different target delivery methods are implemented in the treatment of oral cancers in future. Prebiotics like oligosaccharides have potential in growth enhancement of *Bifidobacteria* and *Lactobacilli* (Gibson et al. 2005) However, several mechanisms of prebiotics involved in oral cancer treatments are still not figured out. Further studies are required to elucidation of mechanisms of prebiotics in the treatment of oral cancers (Table 12.2).

12.4 Postbiotics

Postbiotics are generated through the metabolic activity of the microorganisms which show direct effects on the host (Żółkiewicz et al. 2020). The findings of postbiotics are started by using the cell-free supernatants (CFSs) obtained from bacterial fermentation, primary microbial metabolites (e.g. lactic acids) and short chain fatty acids (SCFA). Also, another important polysaccharide β glucans and odours gas hydrogen sulphide H_2S have properties of both prebiotics and postbiotics (Vrzáčková et al. 2021).

Postbiotics may show anticancer effects by reducing the cell response to metabolites and oxidative stress. Postbiotics are microbial waste products which interrupt the metabolic homeostasis of the host cells by increased oxidative stress. Lactate has the potential to produce mitochondrial reactive oxygen species (ROS) by elevating the $NADH/NAD^+$ ration and used as a metabolite of lactate oxidase (Zelenka et al. 2018). H_2S changes the sulfhydryl entities of proteins and reduces the mitochondrial cytochrome C oxidase increase ROS release (Cao et al. 2019). Also, butyrate and β glucans have properties of enhancing ROS generation in cancerous cells (Vrzáčková et al. 2021).

Postbiotics are also used as adjuvants in the treatment of cancer along with chemo-radiotherapy. Postbiotics adjuvant therapy has associated with the response of the host immune systems. However, the effectiveness of postbiotics is investigated in the gastrointestinal cancer cases. Motevaseli et al. found the antiproliferative properties of various postbiotics *Lactobacillus crispatus* and *Lactobacillus gasseri* derived from vaginal origin on normal and cervical cancer cells (Motevaseli et al. 2013). The most interesting property of postbiotics is

differentiation between normal cells and cancerous cells which leads to proliferation of normal cells and suppression of angiogenesis in cancerous cells (Davis and Milner 2009) Postbiotics like cell-free supernatants *Lactobacillus casei* and *Lactobacillus paracasei* obtained from breast milk are also have anti-carcinogenic effects (Riaz Rajoka et al. 2018).

Other anti-carcinogenic mechanisms of postbiotics reported are decrease in cell viability, stimulation of pro-apoptotic cell death pathways, reduction of microbial translocation, enhancement of apoptosis and necrosis, increase in autophagy and inhibition of cancer cell invasion (Homayouni Rad et al. 2020). Moreover, postbiotics could be an efficient tool for the treatment of OSCC. Further, studies are required to develop potential postbiotics to implement in oral cancer which will reduce the side effects and cost as compared to the conventional therapy.

12.5 Synbiotics

The combination of prebiotics and probiotics are known as synbiotics. Previously, live probiotics are only applicable for the treatment of diseases. But in recent studies, dead probiotics bacteria are also used for the treatment of various diseases. To enhance the properties, live or dead probiotics, prebiotics are used as adjuvants. The major anti-carcinogenic effects of synbiotics are enhancement of apoptotic response to carcinogen-induced DNA damage and increased colonisation, growth, survival and activity of probiotics in the presence of particular prebiotics (Raman et al. 2013). Combination of probiotic strain *Bifidobacterium lactis (B. lactis)* and dietary supplement-resistant starch (RS) interacted with the acute apoptotic response to a genotoxic carcinogen (AARGC). In this study, RS acts as a metabolic substitution for the *B. lactis* to induce pro-apoptotic actions (Le Leu et al. 2005) The anti-carcinogenic mechanisms of synbiotics against cancer cells are described in Fig. 12.2.Thus, synbiotics may exert beneficial effects on the oral cavity which will help in reducing the oral squamous cell carcinoma. Further studies are required to innumerate the beneficial effects of synbiotics in oral carcinomas. Synbiotics also have beneficial effects in combination with conventional chemo-radiotherapy which will reduce the side effects.

12.6 Conclusion

As per the data available based on the in vitro and in vivo studies suggest that probiotics, prebiotics and synbiotics are ideal alternative choice for prevention of oral carcinogenesis. The evolution of molecular techniques and elucidation of oral microbiome, the anti-carcinogenic mechanisms of synbiotics may solve queries of biotherapeutic treatment of cancer. In order to achieve more results in the treatment of oral cancer detailed study and human trials are needed. Synbiotics can be used with caution in immunocompromised patients and contraindicated in new born

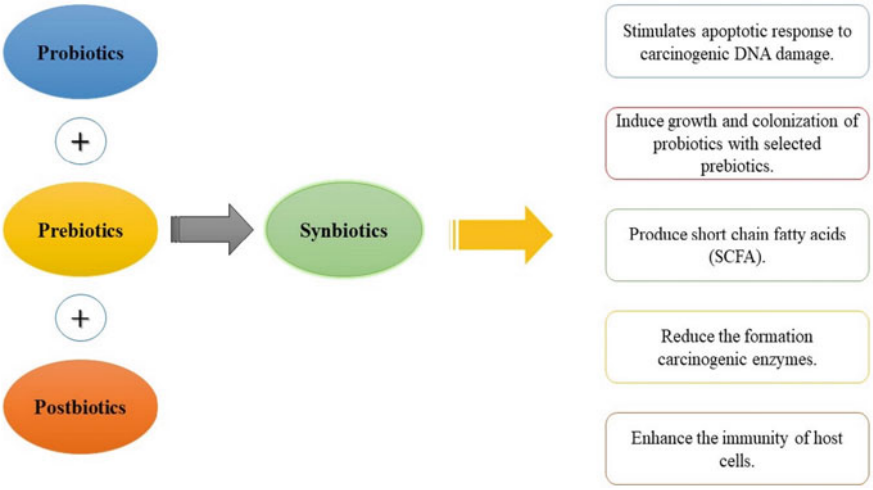

Fig. 12.2 Anti-carcinogenic effects of synbiotics against cancer cells

babies. In conclusion, synbiotics can be a suitable alternative of conventional cancer therapy when administered in required amount.

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Regulatory Issues of Synbiotics in Cancer

13

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13.1 Introduction

Over the previous few decades, cancer has been one of the major causes of death. Despite early detection and treatment, the number of cancer patients and cancer-related fatalities rise day by day. The worldwide cancer burden has increased to 14.1 million in terms of recent cases and 10 million deaths have been reported in the year 2020. Chemotherapy utilizes chemical substances to treat cancer from progress to the level of metastatic stage. The association of natural prebiotics and benefits reveals potential strategies to prevent cancer. The functional meal contains a physiologically active compound, that shows a beneficial effect on human health. Thus, a functional meal helps to prevent, manage, and treatment of various diseases including cancer. The fermented meal comprises the fermented food that contains physiologically active compounds, i.e., probiotics and prebiotics obtained from different sources. The synbiotics also help in the prevention of cancer with added advantages like no potential side effects by the suitable selection of probiotics and prebiotics combination and easy to administer (Thilakarathna and Langille 2018).

Synbiotics are the synergistic combination of prebiotics and probiotics. Probiotics are living and beneficial microorganisms that can live in a healthy intestinal environment. Synbiotics administered in a sufficient amount promote health to the host by encouraging the growth of gut microbial stability. Prebiotics are the non-digestible dietary components and have a favorable effect on the host by provoking certain bacteria in the colon, which helps to improve the health of cancer patients by inhibiting the pro-carcinogen enzyme and inhibit the abnormal proliferation of the cell (Shafi et al. 2014).

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Synbiotics are almost similar to antibiotics that have the probability to manage the infection due to the production of beneficial chemicals by probiotics in the lower intestinal during fermentation. Prebiotics are utilized to boost the probiotic bacterial survival as well as their activity in the large intestine. For the ideal synbiotics product, a synergistic combination should be developed between the beneficial microorganism (probiotics) and selected substrate (prebiotics) and could be obtained from different sources. The appropriate selection of probiotics and prebiotics provides the beneficial combination for the management of various disease conditions such as diabetes, hypertension, allergic viral disease, gastrointestinal disease, and to prevent cancer. The function of the gut microbes as well as the prospective application of synbiotics includes prevention and treatment of cancer with fewer components that impact less number of physiological processes in a targeted way to improve human health (Shafi et al. 2014; Yadav et al. 2010).

13.2 Probiotics

Probiotics are defined as living, non-pathogenic microorganisms that show an advantageous effect on the host. The administration of non-pathogenic microorganisms in a sufficient amount shows an enhanced or beneficial effect on the health and physiology of the host. The most widely used microorganisms that are used for human administration such as Lactobacillus rhamnose, Lactobacillus plantarum, and Saccharomyces Boulardi. Probiotics are introduced into meals that are in fermented form, either it may be single or combination of different non-pathogenic microorganism. For example, a probiotic strain of VLS#3 contains a mixture of eight different kinds of strains. There are many upcoming strains and different kinds of probiotics which prove their application to improve the health of host (Siciliano and Mazzeo 2012; Mariman et al. 2014). The selection of strain should be specific and not generalized. The probiotics may show various beneficial effects when used either alone or in combination with other probiotics. The efficacy of probiotics depends on the patient categories. Only a few studies have been executed to show the potential and beneficial effect of probiotic strain. The choice of probiotics from the different variety of strains generally depends on the producing capacity of lactic acid, non-pathogenicity, adhesive effect on intestinal epithelial tissues, bile and acid tolerability, antimicrobial productivity, robustness, persisting behavior under processing conditions, modulating response on immunity, and inducing capacity of metabolic action (Mariman et al. 2014).

Conservative activity of lactic acid-producing microorganisms prevents the pathogenicity of a large number of microorganisms on account of the formation of fermented by-products such as hydrogen peroxide, diacetyl, and acetic acid. Various by-products are produced by thiocyanate and hydrogen peroxide in the presence of lactoperoxidase enzyme. Another physiologically activated compound is bacteriocin having a peptide-like structure, this bacteriocin shows the bactericidal activity by acting on the specific biological receptor. Apart from that, the variability depends on their distinct chemical constituent and their mechanism of action. Moreover, it

reveals subsequent benefits like modulation of lactose intolerability, management of diarrhea, neutralizing the adverse effect of hazardous chemicals, anti-inflammatory effect on the intestine, preventive activity on the pathogen, and other therapeutic efficacy against liver, vaginal, oral, and mental disorder (Sánchez et al. 2017).

It is necessary to go through several guidelines regarding the selection of prebiotics from the food component such as identification of strain, safety, and functionality of strain, health benefits in humans, straight and non-error labeling of efficacy, and satisfactory shelf life (Sánchez et al. 2017).

13.3 Prebiotics

Prebiotics are referred to as the non-digestible food component such as oligosaccharide which are selectively fermentable and cause the alternation in composition and activity of the intestinal environment and offers certain health benefits to the host. Widely used probiotic are oligosaccharides, xylo-oligosaccharides, fructose, inulin, and other carbohydrates such as pectin, oligosaccharide, soybean oligosaccharide, and polydextrose. However, these carbohydrate-based prebiotics are not present in their purified form, and safety is yet to be evaluated. Criteria for choosing probiotics have relied on the hydrolyzing capacity of the substrate, and absorption through an intestinal epithelial tissue; furthermore, it should be suitable for the intestinal microenvironment (e.g., oligosaccharides must be suited to bifidobacteria). The selected substrate should show an enhanced effect on the luminal environment. These selected carbohydrates possess many properties and therapeutic properties against cancer via stimulating the growth of beneficial intestinal bacteria, altering the expression of genes in the bacterial cell in the colon, cecum, and feces, raising the absorption of micronutrients in a lumen, regulating xenobiotic-metabolizing enzyme, modulation of immunity. Inulin probiotic, a combination of oligofructose with the probiotic Bifidobacterium and Lactobacillus, enhanced anti-tumorigenic activity. Interestingly, a reduction in the pH value of lumen region was observed after the administration of inulin.

The β-galactosidases enzyme produces β (Thilakarathna and Langille 2018; Shafi et al. 2014; Yadav et al. 2010; Siciliano and Mazzeo 2012) GOS by enzymatic transglycosylation activity. The generic formula of the β (Thilakarathna and Langille 2018; Shafi et al. 2014; Yadav et al. 2010; Siciliano and Mazzeo 2012) GOS is (Thilakarathna and Langille 2018; Shafi et al. 2014; Yadav et al. 2010; Siciliano and Mazzeo 2012) [D-Galactose] n-D-Glucose contains 3–10 sugar moieties. The metabolic product of GOS is acetate, lactic acid, short-chain fatty acid, and some other gases. β (Thilakarathna and Langille 2018; Shafi et al. 2014; Yadav et al. 2010; Siciliano and Mazzeo 2012) GOS has various properties and may raise the fecal pH, reduction in nitroreductase activity, regulation of the stool and frequency, and decrease the concentration of secondary bile in feces. Also, lactose and β-galactosidases can prevent cancer (Kaźmierczak-Siedlecka et al. 2020).

13.4 Synbiotics

Therapeutic synbiotics act as a nutritional compound referred to as probiotic bacteria and growth-promoting prebiotic substances that show the beneficial or enhanced effects in combined form. Therapeutically active synbiotics increase the duration of survival of living microbes in the intestine. The main aim of symbiotic to maintain the population of probiotics microorganisms under the abnormal GIT condition like reduced pH, abnormal temperature, and oxygen intolerability. Prebiotics has the potential to induce growth and metabolic activity of probiotics microorganism (Bennett et al. 2004). Cubson and Koilda proposed the two concepts related to the synbiotics viz. complementary concept and synergistic concept.

According to the complementary concept, single or combined forms of probiotic bacteria and their selectivity depend on the desired effect on the host. However, prebiotics can be selected freely to promote the growth of the beneficial microbial population. Prebiotics support the activity and growth of consumed prebiotics in a targeted area. Synergistic concept advocates the selection of prebiotic and probiotic strains according to the survival, growth, and activity of the chosen bacterial strain. The prebiotic component also prolongs the resident time of intestinal microflora (Swanson et al. 2020).

An acceptable combination of synergistic synbiotics should be relevant for single and a mixture of probiotic strains and a combination of prebiotic substrate. Probiotics do not remain alive without the prebiotic substrate in the human digestive system. Basically, prebiotics act as the preservative agent for probiotics. Synbiotics exhibit the anti-carcinogenic effect by promoting apoptosis response by damaged DNA (induced by carcinogens) to the intestine which is caused by the carcinogen. Synbiotics enhance survival, growth, and colonization of probiotics by inducing proliferative activity, increasing the formation of short-chain fatty acid and modulating cyclooxygenase enzyme and NO synthase, i.e., crucial for the development of lumen carcinogen. The right selection of synbiotics regulates the intestinal microbial environment, augments the metabolic activity of the lumen, tones up immunomodulatory responses, and eliminates the risk of cancer. But the extent of safety and compatible performance of synbiotics should be specified. The genomic sequence of probiotics microorganisms must be available to the public to evaluate the gene for safety reasons (e.g., resistance to transferrable antibiotics or production of toxins. The selected strain should be examined by the scientist to proceed with the research and the collection of specific strain must be listed by international cultural organization. The purity, safety, potency, and identity of the living bacteria must be recognized accurately and precisely by a suitable analytical method that follows the regulatory standard according to a category of product (Fig. 13.1) (Raman et al. 2013).

For the identification and selection of another set of synbiotics, the substrate must be chosen according to the utilization of the co-administered bacteria. For the ideal formation of synergistic symbiotic, selected microorganisms should have high growth property.

The main target of the substrate is an ingested bacterium. It is extremely challenging to design and evaluate the potency of synergistic synbiotics. It should be

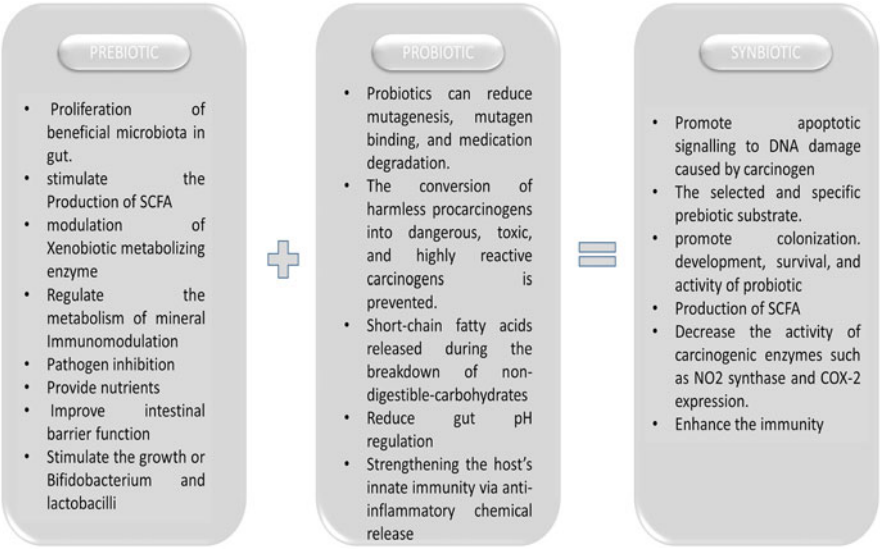

Fig. 13.1 Synergistic benefit of synbiotics

clarified whether the formulation provides the additive activity or not when it is combined. In terms of biology, synbiotics are also used to refer to an environmental relationship. Which tells about the one microorganism lived with another microorganism in the natural ecosystem for a long-term relationship known as "Symbiosis" (Raman et al. 2013).

13.5 Sources of Synbiotics

The ideal formulation of synbiotics depends on the sources and selectivity of prebiotics and probiotics which is obtained from dairy and non-dairy product, fermented product, and aquaculture, and most of the sources are also available in the market. The fermented product helps in the formation of beneficial synbiotics to reduce the risk of cancer and concentration of unwanted metabolites. Basically, the enhanced effect of synbiotics associated with the prebiotics and probiotics obtained from the different sources, either it may be individual or in combination. After the selection of probiotic and prebiotic strains from the possible sources. Synbiotics are formulated according to the compatibility between substrate and living microorganisms. Banrudin et al. formulated a synbiotic yogurt containing the yogurt component with *Lactobacillus helveticus* and prebiotic component polydextrose. Synbiotic yogurt cures irritable bowel disease and relieves constipation. Langa et al. formulated a synbiotic ice cream from the probiotic cheese with *L. paracasei* and prebiotic fructosaccharide. Probiotic concentration was taken at 7logCFU/g which showed several advantageous effects on the health of consumers. Oliveria et al.

formulated a synbiotic fermented skim milk by using a 6logCFU/ml of *S. thermophilus*, 6logCFU/mL of *B. lactis*, and prebiotic inulin (González-Herrera et al. 2021).

Synbiotic non-dairy product is also formulated by using a specific pre- and probiotic strain from non-dairy sources. Pruksarojankal et al. formulated a synbiotic bread by using a probiotic L. casei and prebiotic inulin and glucomannose with the help of traditional method. Waghmode et al. (2020) formulated synbiotic chocolate through the combination of prebiotic flaxseed and probiotic Leuconostoc mesenteroides, which was added into the melted chocolate to form a bar of synbiotic chocolate, and this product demonstrated good antioxidant property (González-Herrera et al. 2021; Jain et al. 2021). Martin et al. isolated the lactobacillus strain from the breast milk of various animal species. The various probiotic strain was founded in breast milk such as *L. gasseri, L. fermentum, and L. salivarius*. This strain stimulates the immune system and provides an anti-inflammatory effect (Lyons et al. 2020).

13.5.1 Prebiotic Sources

Prebiotics are mostly obtained from vegetables, fruits, and legumes. These products are basically enriched with the fibrous prebiotic component obtained from the bananas, chickpeas, kidney beans, onion, navy beans, shallots, leeks, lentils, and so on. Products of the prebiotics are generally classified as an oligosaccharide, sugar alcohol (polyols), and soluble fiber. Extensively used polyols prebiotics are lactitol, mannitol, xylitol, and sorbitol. Most of the prebiotics found from the oligosaccharide contain a mixture of 3–10 sugar moieties with different levels of polymerization. Generally, oligosaccharides are non-digestible and hydrolysable compounds and induce the growth of beneficial bacteria instead of undesirable bacteria. Widely used oligosaccharides which act as prebiotics are isomaltose-oligosaccharide (IMO), xylo-oligosaccharide (XLO), and galactosyl lactose (GL), galacto-oligosaccharides (GOS), palatinose, pyrodextrin, soy-oligosaccharide (SOS), lactosucrose, inulin, and raffinose (Mohanty et al. 2018).

Xylo-oligosaccharide (XOS) is obtained from the fermented meal including honey, fruits, wheat, bran, etc. fructo-oligosaccharide (FOS) widely originated from the plants including wheat, onion, garlic, chicory, Jerusalem, breast milk, sugar beet, etc. and IMO obtained from juice, sauce, miso, and so on. Dietary fiber contains carbohydrate which is a rich source of prebiotics. These dietary fiber are non-digestible agents consisting of different kinds of polysaccharides without containing starch such as beta-glucan, waxes, lignin, and dextrin. Prebiotics are also found in other food supplements with higher capability to stimulate the growth of beneficial intestinal microflora. Other source may include derivative of legacy, lupin kernel fiber, blueberry extract, green tea extract containing selenium, and dragon fruit. Lupin kernel fiber induces Bifidobacterium growth by repressing the number of clostridia (spiroforme, *c. cocleatum*). Most of the functional food also contains the prebiotic agents obtained from bread, soft drink, fruit, drink of lactic

acid, custard, yogurt, cake, sweeteners, sauces, and biscuits. Prebiotics (oligosaccharide) are naturally found in breast milk in 10–13 g/L that enhance the activity of bifidobacteria in infant GIT. Moreover, prebiotics help to prevent the decomposition of food products and keep it fresh and moist for a longer time and improve the quality of product. They are incorporated into the meal and formulated in the form of syrup and powder. Some prebiotic commercial products are available on the market by the name of Ensure® plus fiber (US), fiber gummies sugar-free, and Prebiotin (USA). MS Prebiotic is a prebiotic fiber which reduces the chances of cardio problems and inflammation (Mohanty et al. 2018).

13.5.2 Probiotic Sources

Probiotics are mainly obtained from the dairy and non-dairy food compounds which contain the probiotic strain in large amounts and grow over the duration of time. Dairy product is also available in the market containing probiotic strains like ice cream, yogurt, buttermilk, and non-dairy products including cereals, cabbage, sorghum, soy-based compound, and different types of juices that deliver a sufficient amount of probiotic to the consumer (Kechagia et al. 2013). In addition, another type of food product that is obtained by fermentation of bacteria such as kimchi, olive, pickle, chocolate, beer, bread, and bun. There are different microbial species considered probiotics and classified according to their genus species such as lactic acid-containing bacterial species include lactobacillus, bifidobacterium, and other bacterial species including enterococcus species and saccharomyces species. In

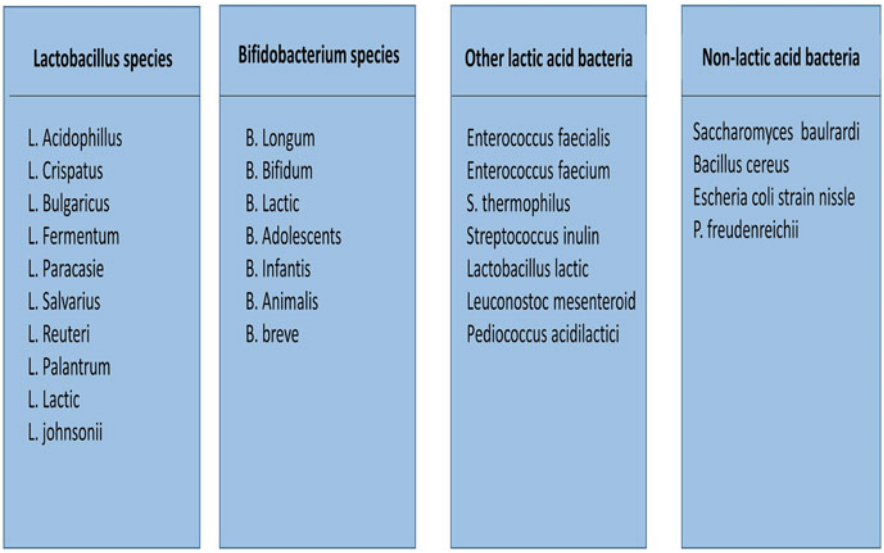

Fig. 13.2 Microorganism considered as a source of probiotic

Fig. 13.2, Gram-negative bacteria is induced by the Gram-positive bacteria, i.e., Lactobacillus species produce the lactic acid which acts as the finished product for fermented carbohydrates. Lactobacillus species include *L. acidophilus, L. gallinarum, L. johnsonii, L. casei, L. paracasei, L. rhamnosus, L. plantarum, L. crispatus,* and *L. gasseri*. Bifidobacterium species use the different metabolic pathways and the species includes in this category is *B. adolescentis, B. breve, B. longum, B. animalis, B. lactis, and B. bifidum*. Some of the other lactic bacteria include *E. faecium, Lactococcus lactic, Pediococcus acidilactici, Sporolactobacillus inulin, Enterococcus faecalis, Streptococcus thermophilus*. Non-acid bacteria include *Escherichia coli* strain nissle, *Bacillus cereus, S. boulardii, Propionibacterium freudenreichii, and Saccharomyces cerevisiae* (Syngai et al. 2016).

In addition, breast milk, GIT of humans, the gut of various animal species includes pig, and poultry, can be considered as source of prebiotic strain. Intestine from the various aquaculture like shrimp, catfish, Carassius auratus gibelio are also a good source of probiotic (Syngai et al. 2016).

13.6 Advantages of Synbiotics Over Other Therapeutics

A large population of cancer patients widely uses the chemotherapeutic agent, radiotherapy, and surgery to treat cancer. Antineoplastic agent such as 5-fluorouracil, mercaptopurine, and methotrexate helps to reduce tumor progression but their administration for a long time, it can cause life-threatening conditions, various adverse and side effects in patients such as inflammatory responses, and alopecia, and inhibits the beneficial bacteria into GIT leading to digestion problems. Administration of synbiotic reduces these side effects in cancer patients and show the anti-tumorigenic effect by inhibiting the conversion of harmless procarcinogen into an active and toxic carcinogen. Synbiotics prevent the overexpression of carcinogens which are responsible for the development of cancer. Probiotics inhibit the carcinogenic agent in GIT as well as inhibit the conjugation of short-chain fatty acid and linolenic acid. Probiotic action helps to decrease the production of the cancer cell and shows an antineoplastic activity by preventing the cell proliferation and induction of apoptotic cell. Pre- and probiotic therapy could be considered prior to and alongside chemotherapy as a method to maintain diversity and promote chemotherapeutic efficacy. One of the studies claimed that synbiotic has the ability to reduce the number and size of the aberrant cyst (Scott et al. 2018).

Antibiotics such as doxorubicin, daunorubicin, bleomycin, and mitomycin were found to prevent the spread of cancerous cells throughout the body and promote cell death, but this antibiotic treatment causes the imbalance in intestinal microflora which leads to diarrheal condition. Diarrhea is the main problem which is mostly associated with antibiotics, and these antibiotics also cause the variation in carbohydrate metabolism, difference in pH, alter the composition of bile acids and short-chain fatty acids. Synbiotics contain specific probiotic strains such as *L. delbrueckii, L. acidophilus, L. fermentum, S. boulardii, and L. acidophilus*, showing the

beneficial effect to reduce frequency of diarrhea caused by antibiotics. Synbiotics have the potential to restore and maintain the microenvironment by reducing the population of the bacteria from GIT. Synbiotics balance the homeostasis, inactivate the pro-carcinogen enzyme, and raise immunity of cancerous patients. All these beneficial effects of synbiotics help to reduce the adverse effect of an antineoplastic agents such as stomatitis, neuralgia, and diarrhea (Motoori et al. 2017).

After the surgery of a cancer patient, chemotherapy is given to the patient to prevent proliferation of cancer cells. The main limitation of chemotherapy is it causes harmful conditions such as diarrhea, reduces the count of white blood cells (febrile neutropenia), and bone marrow suppression. These harmful conditions show the difficulty in chemotherapy and reduce the therapeutic effect of other drugs. Harmful responses to chemotherapy depend on the dosage regimen; higher concentration of dose can produce a higher toxic effect in the cancer patient. For example—patients who suffer from a severe stage of esophageal cancer are typically older and malnourished due to esophageal contraction. Chemotherapy is an extensive initiator to disturb the beneficial bacteria and corrode the intestinal lining. Disturbed microbiota leads to fluctuation in pH of GIT, reduces the inhibition of unfavorable bacteria, and also inhibits the anti-inflammatory reaction. These situations may lead to producing chemotherapy-related harmful effects such as infections and diarrhea. Synbiotics show a significant effect on patients treated with chemotherapy to reduce these chemotherapy-related harmful events. Synbiotic is administered in esophageal cancer patients throughout the time of neoadjuvant chemotherapy to show the positive effect to enhance and balance the intestinal beneficial bacteria. Synbiotic is also advantageous in biliary cancerous patients who go through the hepatobiliary incision and those patients going through the esophagectomy (Batista et al. 2020).

In the case of breast cancer-related lymphedema, reactive oxidative species concentration increases resulting in rise in the level of free radical generation and reducing the antioxidant level. To increase the duration of survival of patient taking chemotherapy, alkylating agent antibiotics, and NSAIDs. These treatment approaches are applied to prevent cancer, but this treatment induces an inflammatory response and shows localized swelling in the body which is caused by an irregular accumulation of lymph in a patient who survived breast cancer. This problem does not associate with the administration of synbiotics. Synbiotics contain the prebiotic and probiotic that balances the gut microbiota which helps to stabilize the microbial environment of GIT, immunity of gut, modulate inhibitory or growth promoter and reduces the disruption of microbiota. Synbiotics have an extensive effect to reduce inflammation and potentiate the innate immunity of the host by the accumulation of anti-inflammatory agents. Some of the synbiotics contain the probiotic lactobacilli that have the antioxidant property, which reduces the risk to increase the reactive oxidative species. Several studies and randomized clinical trials revealed that probiotic microorganisms with the LCD program have antioxidant properties by reducing MDA and increasing SOD enzyme activity. Treatment with synbiotic treatment demonstrated enhanced antioxidant activity with a lesser side effect in patient with breast cancer-related lymphedema (Navaei et al. 2020).

Colorectal cancer (CRC) is a condition that is closely related to chronic inflammatory responses and could be a sign for early stage of cancer development. Some other disease conditions such as inflammatory bowel syndrome, ulcerative colitis, and Crohn's disease can cause colorectal cancer. Patients with IBD have eight- to ten folds of chances of developing of CRC. The anti-inflammatory agent shows an improvement in the inflammatory response and decreases CRC progress. Anti-inflammatory agents such as non-steroidal, anti-inflammatory drugs (NSAID), and COX-2 selective inhibitors show a preventive effect on these responses, but the main problem associated with these agents is that long-term use of these agents can make serious side effects in patients such as allergic reactions, heartburn, erode the lining of the stomach, i.e., it can also cause ulcer, but this problem can be overcome by the beneficial effect of synbiotic with inhibitory effect on tumor production. IBD can be caused by the imbalance of gut microbiota composition and increased concentration of reactive oxidative species by neutrophils that causes the overproduction of oxidants in the colon. Some of the studies reported that a specific combination of synergistic synbiotic is a safe and effective natural therapeutic way to prevent inflammatory bowel disease and also from colorectal cancer. For example—prebiotic natural source *C. tricuspidata* leaf extract combined with *L. gasseri* 505 both substances are fermented with milk to stimulate the growth of these probiotic strains and prebiotic extract. This synergistic synbiotic shows the antioxidant activity through the formation of a specific metabolite, preventing the erosion of the intestinal line without causing a life-threatening condition as well as increases the survival of the patient (Oh et al. 2020). In addition, another example is the probiotic Bifidobacterium animalis combined with resistant starch as prebiotic produces the defensive effect on azomethane-induced rodent cancer model (le Leu et al. 2010). Moreover, a study observed that the appropriate regulation of intestinal microflora can show a beneficial effect to inhibit tumor generation (Genaro et al. 2019).

13.7 Selection Criteria for Synbiotic

Before the formation of synbiotic formulation, the first consideration should be a selection of suitable prebiotics and probiotics which can be consumed independently and have a good influence on the host's health. The appropriate technique appears to be an identification of certain features that a prebiotic has a beneficial effect on probiotics. Prebiotics encourage the growth of beneficial bacteria with no stimulation of other undesired microbes, resulting in a positive influence on health (Markowiak and Ślizewska 2017).

13.7.1 Selection Criteria for Probiotics

According to the recommendation of WHO, FAO, and EFSA (the European Food Safety Authority), probiotic strains must meet both safety and functionality criteria, as well as those related to their technological utility. Probiotic properties are not

connected with species and genus of the microbe but with few and specially selected strains of a particular species. The origin of strain, its lack of interaction with a disease-causing agent, profile of antibiotic resistance contributes the safety of strain. Functional factors are determined by the longevity of the gastrointestinal system and immunoregulation activity of probiotics. Probiotics must fulfil the criteria included in their manufacturing procedure of formulation as well as they should be capable of surviving and keeping their quality retained for a longer time during the storage and delivery. The consistency of effects of probiotics on health should be documented with the characteristics of the strain present in a marketed product. Review articles and scientific research on a single strain might not be utilized for the advancement of another probiotic strain. It has been recognized that, the characteristics of a tested dose of a probiotic strain do not produce the same properties with different dose of the same probiotic strain. In addition, the importance of carrier type is necessary because it can affect the feasibility of a specific probiotic strain, resulting in it can alter the properties of a synbiotic marketed product (Markowiak and Ślizewska 2017).

Prebiotic criterions and their required characteristics

Functionality
• Competitiveness with respect to microbial species inhabiting the intestinal environment. • Capable of remaining viable and retaining their metabolic processes. • Ability to withstand enzyme and bile salts. • Ability to withstand in acidic pH of the stomach. • The endogenic gut bacteria produce bacteriocin resistance and acid. • Inhibitory effect on *Listeria monocytogenes*, *H. pylori, Clostridium difficile*. • Adherence to and capable of settling in a specific area inside the host and must survive for the longer duration of time in the intestinal ecosystem.
Safety
• Originated from animal, human, and fermented dairy products. • Extracted from a healthy person's gastrointestinal track. • Previous record of safety and reliability. • Diagnosis with accurate identification of their genotype and phenotype traits. • No previous data that is linked with infectious disease. • There should be no toxic effect. • Unstable component has an abundance of a gene that causes resistance to the antibiotic. • Lack of ability to degrade the bile salts.
Technological usability
• Culture with large biomass yield, higher productivity, and ease to produce. • Throughout the fixation process (lyophilization), the production and distribution of probiotic goods, survival, and stability of the required properties of prebiotic bacteria should be tested. • In the final product, the rate of survival on storage should be extremely high in aerobic and microaerophilic conditions. • Ensure that the final product has the desired and sensible characteristics. • Stability of gene. • Bacteriophage resistance is a term used to describe the ability to resist bacteriophage.

13.7.2 Selection Criteria for Prebiotic

Wang et al. stated that there are five essential requirements for the categorization of the dietary component. In Fig. 13.3, according to the first requirement, prebiotics should not be digestible or partially digestible from the higher part of the alimentary canal. According to the need of second criteria, they make their way to reach the colon, where they can be preferentially fermented by a possible beneficial bacterium. According to the requirement of the third criteria, fermentation could raise the production or alter the proportion of short-chain fatty acid (SCFA), increase stool bulk, a moderate reduction in the pH of the colon, scale down the fecal enzyme, nitrous fixed product activity, growth of gut bacteria, better immune function which is beneficial for the host. Selective stimulation of growth and/or activity of the intestinal bacteria potentially associated with health protection and well-being is considered other criteria. According to the last criteria, the prebiotic can be endured during the condition of food processing while being intact, incapable of being chemically degraded or unmodified, and ready for the metabolism of bacteria in the colon. Huebner et al. (2008) used different processing settings to examine a variety of widely available products of prebiotics. In varied processing circumstances, they did not discover any significant variability in the activity of the studied prebiotic compounds. Meanwhile, according to Ze et al. using the starch under in vitro conditions can modify the intestinal bacterial ability. Prebiotics should have a well-documented structure and component utilized in pharmaceutical formulation and their food additives must be reasonably simple to get on a larger scale (Wang 2009).

Prebiotics can be substituted for probiotics and could be a supplement to other prebiotics. Prebiotics may compete with different probiotics in respect of their extended stability throughout the shelf life of meals, beverages, feed, chemical, and physical qualities. Other beneficial qualities of probiotics include tolerance against acids, proteolytic enzymes, and bile salts found in the gastrointestinal system. Prebiotic chemicals preferentially encourage the bacteria found in the gut ecology of the host, removing the necessity for bacterial competition. Prebiotics potentiate the gut microbiota, which influences the fermentation action while altering the level of SCFA, providing advantageous benefits to the host. Furthermore,

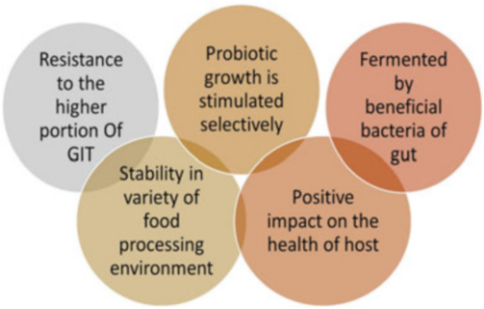

Fig. 13.3 Requirement for selection of prebiotic

prebiotics lowers the intestinal pH and better osmotic water retention in the intestine. Although, taking too many prebiotics can cause diarrhea and gastric problem and cause no such negative effects. Prebiotics can be administered for an extended time and as preventive measures. Furthermore, when the prebiotics are taken in a specific and accurate quantity, they do not produce any side effects like sensitivity to UV light, liver damage, and erosion of intestinal lining caused by antibiotics. Prebiotics are non-allergic and keep a check on antimicrobial-resistant gene in bacteria. However, the effect of the elimination of selected pathogens by the with the help of prebiotics may be inferior to antibiotics. Aside from that, features are listed above form of prebiotic a natural antibiotic substituent (Wang 2009).

13.8 Regulatory Guidelines for Synbiotics

The term synbiotics was discovered some years ago; there was not much conceptual clarity recording the term. It was thought that synbiotics were a combination of prebiotic and probiotic that had a positive effect on the host; according to FDA (food drug administration) and ISAPP (international scientific association for prebiotic and probiotic), symbiotics are defined as "a mixture consisting the of the live organism and substrate selectively utilized by host microorganism that confers a health benefit on the host" (Hill et al. 2014).

ISAPP gave the term symbiotic; an export panel consisting of the academic scientist was brought together. A meeting was held among them and have shared their conclusion about the symbiotic. Under this term, host microorganisms constitute autochthonous and allochthonous microbes. Either of them can be targeted by substrate contained in the symbiotic to a subcategory of symbiotic were defined as complementary and synergistic. When the substrate is designed as selectively administrated or utilized by microorganisms is called a synergistic synbiotic. A complementary synbiotic in which a prebiotic is combined with a probiotic is explicitly designed to target autochthonous (external) microorganisms. A synbiotic can be used in intestinal or extra-intestinal microbial environments, and they use a wide variety of products such as drugs or nutritional supplements, foods, non-foods, and feeds. The effect of synbiotics has been beneficial to the health of animals, humans, subpopulations, and agricultural species. According to ISAPP, the use of synbiotics as a health supplement is safe and has high selectivity to the host organism, which improves the host's health.

Product safety and product labeling are the two main concerns of regulatory bodies. These include both honesty and conformity with the help of regulatory legislation, even if the term "synbiotic" isn't widely used. The guidelines or regulations are incorporated in government guidelines or regulations. Our recommended scientific meaning will define the term. Assist with regulatory control of synbiotic goods (Gibson et al. 2017).

Different regulatory bodies will have different regulatory rules, geographical locations, regulatory classifications, types of allowed claims, and premarket approval. Furthermore, additional production, efficacy, and safety standards exist

depending on the geographical region and product type. The term "synbiotic" is ambiguous. Those would be the requirements for a symbiotic. Things are relevant to the class (e.g., a drug, a food, or a beverage) (Swanson et al. 2020; Gibson et al. 2017).

In areas where probiotic-specific rules are in place, regulatory problems might arise. In addition, there is a proposal 88 in the works. Codex Alimentarius is considering a proposal that might result in worldwide probiotic-specific standards. The Codex is a set of rules that governs how Alimentarius is a set of rules and recommendations for food safety and rules of conduct developed under the auspices of the United Nations Food and Agriculture Organization. The United Nations and the World Health Organization have joined forces to safeguard consumer health and promote fair food trade practices. Probiotics may be affected by Codex Alimentarius requirements.

The European Union has concluded that labeling a food product accounts to an implied health claim, one regulatory consequence of probiotic and prebiotic classifications. The use of "probiotic" or "prebiotic" on a food label is subject to a health claim in the European Union because health claims must be approved procedure approval even though there is some dispute about this circumstance. We may expect the European Union to take action. Adopt a similar stance regarding the term synbiotic because it necessitates proof of health benefit (Swanson et al. 2020; Hill et al. 2014; Gibson et al. 2017).

13.9 Characterization of Synbiotic

Characterization must be carried out to ensure the safety and active performance of symbiotics. The genome sequence of live microorganisms present in synbiotics should be available to the public. The gene safety must be assessed for the production of toxins and to find out the possibility of any transferrable antibiotic resistance. For their easy identification, microorganisms should be named as per the current taxonomy. The identity, purity, safety and potency of the microorganism should be defined properly to meet regulatory standards. The structure and purity of substrate should be characterized by accurate chemical analyses. The contamination must be identified and characterized as per regulatory norms. The level of purity is needed to confirm the consistent performance. The percentage of commercially available prebiotics ranges 35–99% (Srivastava and Mishra 2019; Porras-Domínguez et al. 2019; Crittenden and Playne 1996). After oral administration, the carry-over of monosaccharides and disaccharides produced during the production are digestible and absorbed from the upper gastric tract of the host. The monosaccharides and disaccharides carried over from the production process that are present in prebiotic preparations are typically digested and absorbed by the host in the upper gastrointestinal tract after oral ingestion. The selection of specific microorganism, type and quantity of materials for the production of formulation is a critical aspect and cannot be ignored due to effects on the health of target host. For example, only 3 g of active substrate will be obtained from the 6 g dose of 50% pure galactooligosaccharide.

Furthermore, the active ingredients of synbiotics must be sufficiently stable. However, the stability of live component is challenging (Jackson et al. 2019). The viability of microorganism is highly dependent on matrix (dried, liquid or ointment), pH, oxygen, and temperature. The shelf life could be short in case of liquid symbiotic product and ranges 1–2 weeks, while the shelf life may be extended up to 2 years in case of encapsulated or lyophilized powder (Swanson et al. 2020).

13.10 Safety Measures for Synbiotics

However, both probiotics and prebiotics showed safety to date (Closa-Monasterolo et al. 2013; Olesen and Gudmand-Hoyer 2000; Lasekan et al. 2015; van den Nieuwboer and Claassen 2019; Sanders et al. 2016; Sanders et al. 2010). On the basis of the safety of prebiotics and probiotics, the symbiotic formulation can be presumed safe for the intended use. But any novel formulation must be evaluated to ensure its safety. Unfortunately, no information is available regarding the adverse events (AEs) caused by pre- or probiotics. The reason for no availability of AEs is that either they are considered as inherently safe as food ingredients or due to failure to comply with norms for reporting harms in randomized clinical trials (RCTs). Nonetheless, clear guidance for reporting AEs and serious AEs is provided by CONSORT (Ioannidis et al. 2004) and these standards should be followed. Describing such events as "unrelated to the study product," without justification for this statement, is unacceptable. A systematic review of 384 studies of pre-, pro-, and synbiotics concluded that no safety-related data reported or only generic statements described (Bafeta et al. 2018). Unfortunately, no definition or serious AEs, number of AEs, and withdrawal of participants due to AEs were provided (Swanson et al. 2020).

13.11 Factors to Be Considered for Research

13.11.1 Trial Design

Placebo-controlled, double-blind, and randomized trial can be considered, and trials should be registered as per protocols. Randomization, appropriate blinding, inclusion or exclusion criteria, and sufficient statistical powers were used to analyze the primary outcomes from each treatment group. However, these factors are not limited. Crossover design can be considered for the study of gut microbiota. The wash-out period must be decided on the basis of primary outcomes with consideration of secondary outcomes. In the case of gut microbiota, 2-week washout between condition is quite enough. A longer wash-out period could be considered in case of participants with constipation or other functional bowel disorders. For long-term study parallel-arm design is preferable. The number of study groups can vary to find synergism. Demonstration of selective utilization of substrate by microbiota and

health outcome must be in the same study. In case of failure of experimental clinical trials, observational trials could be a choice.

13.11.2 Selection of Participants or Population

The selection of population can be decided on the basis of the selection of species including non-human species, life stages such as infants, children, adults, elderly or pregnant women, and health status like healthy individuals, at risk, or diagnosed with disease conditions. Diet and medication disturbing microorganisms can be considered as eligibility criteria (Meance et al. 2003).

13.11.3 Intervention

Full description of the intervention is recommended for the ease of replication of the study. Description of intervention includes description, the dose of the pre- and probiotics, strain, time and route of administration, structure, and purity of substrate. Diet intervention should follow the validated methods established by National Cancer Institute Dietary History Questionnaire and Automated Self-Administered 24-h dietary assessment tool (ASA24). Also, nutrient analysis software, i.e., Nutrition Data System for Research can be used.

13.11.4 Selection of Placebo or Control

Generally, complete inert control is chosen. A low dose of highly digestible saccharides such as corn starch or maltodextrin or microcrystalline cellulose (low digestible) are acceptable. Moreover, these placebo enable double blinding.

13.11.5 Outcome

Health and microbiota outcomes should be studied in same study. The primary and secondary outcome must be defined clearly. The outcome hypothesis should provide a clear insight microbiota-driven mechanism for the health outcome. Viability, abundance, change in population, composition of administered microorganism, and metabolites produced by microbiota must be included.

13.11.6 Statistics

The sample size should be adequate for a specific outcome by using intention to treat analysis. Mediation analyses could be preferred to evaluate the beneficial effect of microorganism on health. Ancillary analysis may include responder versus

non-responder, population subgroups, exploratory microbiota analysis (Moher et al. 2012).

13.12 Conclusion

A synbiotic is a type of mixture comprised of prebiotics and probiotics and is beneficial for host health. The selection of prebiotics and probiotics must be specific and precise to avoid any deter effect, the possibility of transfer of resistance gene to the bacteria, etc. However, no proper updates regarding the adverse events are available, and more studies or clinical trials are necessary for their successful clinical applications. The selection of substrate, microorganism, and dose must be defined after adequate study or as per the best information available. The various available guidelines may help researchers in designing novel synbiotics to treat ailments and diseases or other terminal illnesses such as cancer.

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Synbiotics in the Management of Breast Cancer

14

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14.1 Synbiotics

Many studies have been conducted over the last few decades to prove pro- and prebiotics' health benefits and therapeutic potential. It has been shown that functional meals alter, adapt, and heal pre-existing gut flora. They also help to keep the intestinal ecosystem in good working order. When Gibson first proposed the concept of synbiotics, he hypothesized the added benefits that could be obtained by combining prebiotics and probiotics to produce synbiotics. Prebiotics are the substance obtained by dietary health beneficial food which are utilized by host intestinal bacteria for their fast-growing health. In other words, prebiotics are the diet or fuel for the intestinal flora which support fast growth for health benefits. The most often used prebiotics include fructooligosaccharides, galactooligosaccharides, xylooligosaccharides, inulin, and fructans, which when combined with probiotics like *Bifidobacterium, Lactobacilli, S. boulardii*, and *Bifidobacterium* coagulant

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could improve microbial viability which is beneficial for improving the immunity and support to prevent the mucosal damage and prevent the invasion of pathogenic microorganism into GIT lining. The synbiotics were developed to conquer the challenges of probiotic survival in the GIT. Synbiotics improve health conditions by providing necessary healthy supplements to GIT.

Evidence of enhanced probiotic bacteria survival throughout upper digestive tract transit tends to support the use of synbiotics. The stimulating effects of probiotics and common bacteria, as well as more efficient colon implantation, all help maintain intestinal homeostasis and a healthy body. The probiotics are administered by oral route in the form of fine granules and suspension form. It can be given in single or multi-strain combinations. They are remaining intact in the upper part of the GIT system, but when they reached the lower part of the GIT system which is their favorable growing environment then they grow very fast. They have the capacity to neutralize the toxin excreted by pathogenic microorganisms (Scott et al. 2018). They are fast growing and not given chances to grow other types of pathogenic microorganisms. Due to their fast growth, they make competitive expulsion to the pathogenic strain of microorganisms from the GIT. It can antagonize the cancer-causing agents and reactive oxygen species or free radicals. It promotes the metabolism of fatty acids and the biosynthesis of vitamins by fermenting the nutritional food in the intestine. It helps to the expulsion of gases by increasing the peristaltic movement of GIT (Hill et al. 2014; Dimidi et al. 2017).

The benefits of consuming synbiotics include:

1. Balanced gut microbiome with increased *lactobacilli and bifidobacteria* counts.
2. Symbiotic treatment for 12 weeks decreased IHTG concentration in non-alcoholic fatty liver disease patients (Eslamparast et al. 2014).
3. Improved immuno-modulating ability (Cazzola et al. 2010).
4. symbiotic therapy (*L. casei, S. thermophilus, L. acidophilus, B. longum, L. bulgaricus*) for 28 weeks lowered fasting blood sugar levels and insulin resistance (Rahimi et al. 2022).
5. In surgical patients, the symbiotic treatment prevented bacterial translocation and decreased the frequency of nosocomial infections (Li et al. 2021).
6. *L. rhamnosus GG, B. lactis* Bb12 and inulin treatment for colon cancer patients resulted in more *L. rhamnosus and B. lactis in faeces* and decreased IL-2 secretion (Rafter et al. 2007).
7. Weight loss and leptin decrease were seen after treatment of *L. rhamnosus* and inulin along with a rise in *Lachnospiraceae* in a pool of 153 obese persons (Sanchez et al. 2014).

14.2 Synbiotics in Cancer

In the preceding decade, much has been written about the significance of microbiota–host interactions in cancer plasticity and the development of cancer in humans. In dysbiotic environments, pathogenic microbes outnumber or replace

non-pathogenic ones, resulting in altered/disturbed physiological systems and leading to various disorders, including cancer. Pathogenic microbes appear to cause epithelial barrier disruption with epithelial-mesenchymal transition (EMT) activation and inflammation to induce carcinogenesis. So, restoring the healthy physiologic microbiota using symbiotic treatment has become one of the revolutionary anticancer therapeutic strategies (Vergara et al. 2019). It was investigated from a previous study that cyclophosphamide is an anticancer drug and has microbiota-mediated induction of anticancer Th-1 and Th-17-mediated immunity against colorectal cancer cells. Without microbiota, cyclophosphamide is unable to show its anticancer effect and cancer cells develop resistance against cyclophosphamide (Long et al. 2019). In another study, it was found that the anticancer effect of cisplatin is suppressed against skin cancer due to oral antibiotic administration. Oxaliplatin works with reactive oxygen species and antibiotics kill all the microbiota of the GIT system and reduce the reactive oxygen species gene. Without microbiota, antibiotics block the anticancer effect of oxaliplatin (Iida et al. 2013; Perez-Chanona and Trinchieri 2016).

14.2.1 Inflammation-Induced by Microbiota

The host's ability to manage inflammatory responses in various bodily regions is fundamentally linked to the microbiota's role in the development of cancer. IFN levels were shown to rise in association with Streptococcaceae species like *Streptococcus australis* and *Streptococcus parasanguinis*. A particular microbiota's production of numerous inflammatory cytokines may harm DNA in a variety of ways, including aberrant DNA methylation. In the long run, cancer may result from this DNA damage. In the presence of commensal microbes, TLR5 increases the regular secretion of IL-6 and inflammation, which then promotes the growth of tumors. Long-term inflammation may also result in dysbiosis, altering the composition of typical flora and increasing the chance that some bacteria with genotoxic properties may proliferate, creating the ideal conditions for cancer. By encouraging the development of myeloid and c T cells, the microbiota causes the lung parenchyma to release IL-1, IL-23, and IL-17. By raising the host's production of IL-1 and TNF, Gram-negative bacteria like *Helicobacter pylori* aid at the beginning of inflammation-dependent carcinogenesis. *B. fragilis* causes an inflammatory cascade in the intestinal mucosa via IL-17R and Stat3.

This results in the formation of a CXCL1 that promotes the development of immature myeloid cells to support colorectal cancer. Furthermore, Lactobacillus spp. infection of the urogenital tract results in IL-2 and IL-4 level enhancement as *F. nucleatum* will enhance the biosynthesis of Tumor Necrosis Factor-α, Interleukin-6, Interleukin-8, and Interleukin-1. EMT activation in most cancer cells is like ordinary tumor-related macrophages that promote cytokine secretion and extracellular matrix alteration. TAMs produce soluble increase elements together with HGF, EGF, TGF, and PDGF, in addition to inflammatory cytokines together with Interleukin-1 and 6, and Tumor Necrosis Factor. Chronic immunosuppression with

the aid of using regulatory dendritic cells and T cells in reaction to irritation is connected to EMT (Vergara et al. 2019).

Most of the research for probiotic interactions with the host in tumor proliferation has focused because the large intestine has a much more diverse and large number of microorganisms (10^{12} bacteria/g stool). It was found from previous research that some bacteria's got growth about ten times more as compared to normal bacterial content. And some pathogenic bacteria at elevated levels decrease the immunity of the body against the cancer cell. It also alters the metabolism, increases the level of toxins, and increases the invasion by increasing the permeability and inflammation in the GIT system. It also alters the blood and lymph flow system. On another side, some microbiota may be induction of IL-18 in the large intestine reducing the chance of colitis and colorectal cancer. Sethi et al. 2018 studied that the use of antibiotics during cancer therapy increases tumor regression due to an increase in immunity. It is because of the high production of T cells producing IFN- γ and reduced production of T cells which is responsible for producing IL-17a and 10. High immunity supports the anticancer activity of chemotherapeutic drugs (Wroblewski et al. 2010).

14.3 Microbiota of GIT

14.3.1 Oral Cavity

In the mouths of healthy people, a variety of species of microbiota live in harmony with the host and exhibit a balanced immunoinflammatory condition. An unhealthy lifestyle increases the risk of gum disease. *Porphyromonas gingivalis*, a keystone pathogen in the ectogenesis of gum diseases, facilitates dysbiosis through modifying the intra- and extracellular environment and releases dangerous signals to produce cytokine and other factors which can induce inflammation in the oral cavity. The molecular pathomechanism related to *Porphyromonas gingivalis*-dependent progression of squamous cell carcinomas is well known. It retards the apoptosis in epithelial cells through PI3K/Akt signaling. *P. gingivalis* support overexpressing the CD44 receptor on the tumor cell which results in the fast growth of oral cancer.

14.3.2 Microbiota in Stomach

Helicobacter pylori causes bacterial dysbiosis in the stomach and is a major cause of gastric cancer. *Helicobacter pylori* colonizes the human stomach, causing a complicated inflammatory and immunological response, which includes the generation of TNF and IL-1, which inhibit gastric acid production. This sets off a chain reaction that begins with chronic gastritis and slowly progresses to atrophy, intestinal metaplasia, dysplasia, and mortality (Wroblewski et al. 2010). *H. pylori* infection can promote the growth of other pathogenic bacteria that release toxins and other immunogenic factors, and support increases the invasion and interferes with the signaling pathway of epithelial cells resulting it may provide chances of

development of stomach cancer. It supports VEGFR-mediated angiogenesis in the tumor (Ohno and Satoh-Takayama 2020).

14.3.3 Microbiota in Colon

Interactions between bacteria and their hosts and microbial interactions in the gut may have a major impact on the onset and progression of CRC. *Escherichia coli, Bacteroides fragilis*, and *Fusobacterium nucleatum* appeared to have a vital role in developing colorectal cancer. A complex community of cancer cells, immune cells, and numerous microbes should exist in the tumor microenvironment of colorectal carcinoma (Kosumi et al. 2018). Influences of *F. nucleatum* on colorectal carcinogenesis: From the rectum to the cecum, the proportion of *F. nucleatum* increases linearly with stages of cancer. Enriching *F. nucleatum* causes high microsatellite instability, advanced disease stages, and low T-cell infiltration levels in cancer tissue. This is a potential therapeutic approach that specifically targets bacteria *F. nucleatum* in colorectal cancer. Another important pathogenic bacteria related to CRC is *Bacteroides fragilis (B. fragilis),* it is hardly detectable in the normal gut microbiota. In order to colonize the intestinal tract, the enterotoxigenic B. fragilis produces a biofilm that can stimulate the production of COX-2, which releases PGE2 and sets off a chain of inflammatory responses. Additionally, *B. fragilis* breaks down E-cadherin, which causes an increase in spermine oxidase, which causes DNA damage and the start of carcinogenesis. A patient with adenomatous polyposis disease family history shows a high level of tumor load in the colon. Bacterial adhesion to the mucosa layer is increased by adenomatous polyposis disease. *B. fragilis* and the mutant-APC gene genotype further increase the risk of CRC development (Cheng et al. 2020). In the IL-10-deficient mouse model, the microbial state influenced the development of colitis-associated CRC. Alterations in the intestinal microbiota's diversity and composition, including a steady decline in richness and diversity and an increase in *Proteobacteria* and *E. coli* at the beginning of inflammation in IL-10-deficient animals (Maharshak et al. 2013). A recent study examining the alterations in the intestinal steroid profile of IL-10-deficient animals during the development of colitis found that the microbial diversity was lower than in wild-type controls, and *E. coli* and *Enterococcus gallinarum* were more prevalent (Keubler et al. 2015). Therefore, it is thought that the significant loss of bacterial diversity is not only a major element in the pathogenesis of IBD but also a contributing factor to the development of colitis-associated CRC in the IL-10-deficient animal (Xue et al. 2017).

14.4 Anticancer Mechanisms of Prebiotics and Probiotics

The symbiotic treatment helps to overcome intestinal barrier loss, produce immunomodulation, elevate the SCFAs, and prevent proliferation (Fig. 14.1). The role of pre-, pro-biotics in cancer treatment is well established. But studies related to

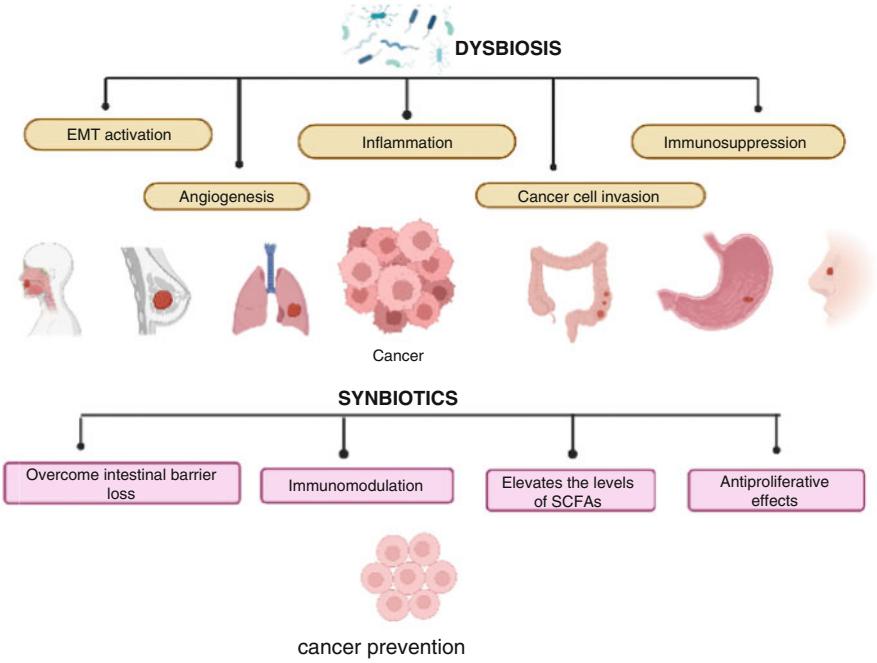

Fig. 14.1 The different ways through synbiotics help in cancer treatment

synbiotics, and cancer prevention are still in the initial stage. Its role in cancer prevention is explained below:

14.5 Microbiota in Cancer Prevention

In a randomized, double-blind, placebo-controlled trial study, symbiotic (oligofructose-enriched inulin + *Lactobacillus rhamnosus* and *Bifidobacterium lactics*) administration in polypectomized patients and patients with CRC, resulted in a significant increase in *bifidobacteria* and *lactobacilli* and a decrease in *Clostridium perfringens* (Roller et al. 2004). In polypectomized patients, the synbiotics significantly reduced colorectal proliferation and the ability of fecal water to induce necrosis in colonic cells, as well as improved epithelial barrier function (Kosumi et al. 2018). Furthermore, synbiotic consumption reduced IL-2 secretion by peripheral blood mononuclear cells in polypectomized patients while increasing IFN-g production in CRC patients (Fotiadis et al. 2008; Gavresea et al. 2018).

The use of probiotic bacteria imparts major support to developing immunity and anticancer character directly and indirectly. Some bacterial strains can degrade the carcinogen and toxin present in the biological system. The study showed that delivery of *Bifidobacterium adolescentis* reduces the cancer cell progression in

Caco-2 and HT-29 cancer cell lines (Kim et al. 2008). *Lactobacillus fermentum* was found to reduce up to 23% of cancer cell progression (Thirabunyanon et al. 2009). In another study Bacillus, polyfermenticus reduced cell growth of HT-29, and Caco-2 cancer lines. It was found from the study *that Lactobacillus rhamnosus* GG increases the programmed cell death in Caco-2 cells (Altonsy et al. 2010).

A study was carried out using *Bacillus polyfermenticus* strain and checked its efficacy to retard the growth of the NMC460 cancer cell lines. The study found it retard the colony formation in cancer cell line culture (Ma et al. 2010). *Lactobacillus paracasei*, *Pediococcus pentosaceus*, and *Lactobacillus plantarum* also reduced the cell growth of cancer lines (DLD-1 and Caco-2) (Orlando et al. 2012; Thirabunyanon and Hongwittayakorn 2013; Sadeghi-Aliabadi et al. 2014; Górska et al. 2019; Hill et al. 2014).

14.6 Microbiota in Cancer Progression

Many experimental data correlated between dysbiosis and cancer formation in regions other than the stomach have been collected, including the oral and nasal, lungs, skin, and breast. It was also found that from a previous study some stains of microbiota bacteria support tumor growth. It induces inflammation in the GIT which is responsible for the tumor progression. Arthur et al. 2012 effect of inflammation on cancer progression. It was concluded from the study that inflammation can modify the GUT microflora and it can promote colorectal cancer. It increases the level of interleukin 10 (IL10). Ray et al. Ray 2012 also studied that high inflammation promotes the level of IL10 which supports colitis. The study was subsequently followed by the administration of azoxymethane (carcinogenic substances) which develops colorectal cancer in animals rate of colitis.

Sánchez-Alcoholado et al. 2020 studied the effect of obesity and gut microbiota concentration in the development of colorectal cancer. In the study, the author investigated the concentration of microbiota in the stool of colorectal cancer subjects with and without obesity. The author found that obesity is not a major cause of cancer, and it changes the level of specific gut microbiota content. But colorectal cancer patient with obesity reflects the specific GUT microflora which was confirmed by the low level of butyrate generating microorganism and increase the pathogenic microorganism increases the level of IL-1β which are responsible for GUT permeability may cause colorectal cancer. A similar study was done by Cani et al. 2009 in which gut microbiota increase the level of glucagon-like peptide-2 in an obese rat model which increases the GUT permeability and may increase the cause of colorectal cancer.

Cell line and the in vivo study showed that *Fusobacterium nucleatum* has induced the inflammation due to the adhesion of this strain of bacteria to epithelial cadherin which supports the signaling pathway of β-catenin that results in inflammation which supports carcinogenic substances to promote cancer development (Gupta et al. 2022). Yachida et al. 2019. Rubinstein et al. 2013). It was revealed that *Fusobacterium nucleatum* created chronic infection and its cell wall component

interacted with immunological factors that result in a decrease in immunity and an increase in the level of invasion of antigenic factors which support the carcinogenesis (Gholizadeh et al. 2017; Long et al. 2019).

In another study it is found that *P. anaerobic* bacterial strain promotes the progression of tumor cells. After attaching to the tumor cell, it acts with α_2 and β_1 integrin of normal cells of the colon, then it results in the activation P13K-Akt signaling which increases the inflammation and tumor cells growth (Long et al. 2019). Dai et al. 2019 discussed that microbiota supports the progression and growth of colorectal cancer. Research explores the different signaling pathways which support the inflammation due to microbiota presence. The toxin and immunogens secreted by microbiota can cause the progression of cancer. Some highly pathogenic anaerobic and aerobic bacteria such as *fusobacterium nucleatum*, *Porphyromonas gingivalis,* and *Parvimona* can be responsible for the development of cancer. An example of human *papillomavirus* is well known to develop cervical cancer. The GIT parasites and fungus are also reported to facilitate the growth of cancer cells (Sun et al. 2020; Mirzaei et al. 2021; Zhao et al. 2021).

14.6.1 Microbiota in Prevention of Intestinal Barrier Loss

The complicated and cooperative relationship between impediment decline, carcinogenesis, and inflammation is elucidated by Mucin 2 homozygous mutation rodents that do not generate stomach muck to function as an impediment and spontaneously achieve colon malignancy. Patients accompanying ulcerative colitis have an injured barrier function, which raises their chances of expanding CRC. Synbiotic situation of *Lactobacillus gasseri* and *Cudrania tricuspidata* leaf extract in fermented milk stated an increase in mRNA and protein levels that help to upgrade hurdle loss and restore the mucus coating and tight connection in azoxymethane persuaded colitis-joined colorectal tumor rodent model. Zhigang Xue and others transported a study to figure out the belongings of synbiotics on intestinal obstacle function, and their preference over probiotics and prebiotics in rodent models. The report replies to probiotics can increase the colonic probiotics, while synbiotics can increase probiotics aggregation, enhance mucosa thickness in the colon, and decrease lactulose/mannitol percentage and bacterial switch. Synbiotics combination overcomes intestinal mucosal impediment deficit in rodents afterward cecectomy and gastrostomy (Moser et al. 2019. A study attended in IBS-D patients following in position or time-spoken presidency of the synbiotic mixture (prebiotics grain vigor, maltodextrin, inulin, fructooligosaccharides, potassium chloride, magnesium sulfate, enzymes and 7.5 × 109 of the following probiotic bacterial strains: *Lactobacillus casei* W56, *Lactobacillus acidophilus* W22, *Lactobacillus paracasei* W20, *Lactobacillus salivarius* W24, *Lactobacillus plantarum* W62, *Lactococcus lactis* W19, *Bifidobacterium lactis* W51 and W52, and *Bifidobacterium bifidum* W23).

Revealed the influence of cooperative situation in elevating the mucosal microbial difference, the colonic CD4+ T cells, the polluted acetate and butyrate levels and

a decrease in *fecal zonulin*, and expediency of gut impediment function (Mirzaei et al. 2021).

14.6.2 Immunomodulation

The key effectors in the recognition and destruction of cancerous host cells are dendritic cells, natural killer (NK) cells, and T cells. By interacting with accompanying dendritic cells by way of cell-surface pattern identification receptors specific toll-like receptors, probiotics advance T and NK cell answers. In a study administered by Le Leu and others, the severe apoptotic response to azoxymethane was considerably raised by *B. lactis* in a synbiotic blend with antagonistic sugar. It seems that renovation of the number of CD8-positive T lymphocytes played a key act. In two together experimental subject and human studies, synbiotic and probiotic formulations including *Lactobacillus casei* or *Bifidobacterium lactis* have continued to display increased NK cell action. Rodent studies have shown that probiotic-inferred NK cell stimulus inhibits tumor growth. The synbiotic situation of oligofructose-improved inulin accompanying *Lactobacillus rhamnosus* and *Bifidobacterium lactis* increased NK-cell action that is suppressed in azoxymethane. By increasing phagocytic project, bearing IgA, stimulating T and B cells, and changing the physicochemical environments of the colon by upsetting pH, synbiotics advance the host immunological reaction (Scott et al. 2018; Pandey et al. 2015; le Leu et al. 2010).

14.6.3 Antiproliferative Effects

Rats fed with resistant starch and *B. lactis* had a lower incidence and multiplicity of colonic neoplasms; the underlying mechanism is that resistant starch acts as a metabolic substrate, helping the probiotic species to perform optimally. Probiotic strains have been shown to have antiproliferative and pro-apoptotic characteristics. *Lactic acid* bacteria have been demonstrated to trigger or promote apoptosis in cancer cell lines, with comparable results observed in animal models. Several mechanisms have been proposed to explain these effects.

14.7 Role of Microbiota in Breast Cancer

14.7.1 Microbiota in Breast

It is revealed from previous research that in the case of breast cancer the microbiota is found in very low quantity which may conclude that the risk of breast cancer is increased in the absence of cancer. Microbiota in sufficient concentration may prevent the progression of cancer in the breast by minimizing the chances of invasion by boosting the immunity, and inflammation and releasing some factors which

support the anticancer activity of the therapeutic molecules. Generally, breast cancer is more common in women, and it is the second most common type of cancer globally and also responsible for the second cause of death due to cancer. There so many factors are available which may, directly and indirectly, affect cancer therapy. The use of antioxidants and probiotics is very common to synergize cancer therapy. Pre- and probiotics can play a very important role in the prevention and progression of breast cancer. The health supplement provided by the pre- and probiotics is very helpful to maintaining health and also for the prevention of cancer progression.

The healthy breast microbiota pattern consists of Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes in decreasing order. *Methylobacterium radiotolerans* were detected in comparatively high concentrations in estrogen-positive tumor tissue, whereas *Sphingomonas yanoikuyae* was found in substantially higher concentrations in paired normal tissue. But, when compared to healthy and early-stage BC tissue, the total bacterial DNA load in cancer tissue was reduced. Breast cancer patients had higher numbers of *Enterobacteriaceae* and *Staphylococcus* germs. DNA double-strand breaks (DSBs) were created in breast cancer patients' normal surrounding tissue by *Escherichia coli* and *Staphylococcus epidermidis* bacteria. DSBs are the most damaging sort of DNA damage. The presence of Proteobacteria in breast tumor tissues was discovered utilizing RNA sequencing data from 668 breast cancer samples and 72 non-cancerous adjacent tissues from the TCGA dataset. Actinobacteria, on the other hand, predominate in non-cancerous tissues. A study found that a different strain of microbiota was present in the breast microbiota present in the different women of different regions. Canadian and Ireland women were found with proteobacteria.

14.7.2 Microbiota Dysbiosis in Breast

The gastrointestinal (GI) tract is the main dwelling area for various microbial communities such as viruses, fungi, and bacteria. They are mainly present in higher percentage in the upper part of the gut, which includes the stomach, duodenum, and jejunum while in the lower part of the gut, ileum, and proximal region of the colon, the presence of these microorganisms is less. These microbiotas are performing diverse functions including essential vitamin production, metabolites synthesis, assistance in the digestion of food, detoxification, drug metabolism, and maintenance of GI physiological homeostasis. Two-way communication between host and gut microbes is important and able to affect several biological activities/systems associated with regulating metabolism as well as immunity to affect the host's health in a negative and positive way (Singh et al. 2021; Fan et al. 2021).

The terms "microbiota" and "microbiome" are used synonymously. In a more precis way, the term microbiota is used for the assemblage of microorganisms in a particular environment, like the colon, upper gastrointestinal tract, middle meatus, saliva, subgingival, bronchial wash, sputum, and throat. Microbiomes refer to the entire habitat of a host region with its surrounding environmental conditions (Martinez et al. 2021; Sirisinha 2016).

Dysbiosis, also known as gut microbial imbalance, is a condition of core pathophysiology affecting the GI motility and causes metabolic disorders, which include inflammatory bowel disease, irritable bowel syndrome, cardiovascular problems, diabetes, and others. Dysbiosis condition includes an increase in small bowel bacteria, conversion of benevolent microbes to pathogenic ones, the translocation of colonic bacteria, etc. The various factors are coupled and play a key role in the development of disease continuing its progression to further critical levels for various diseases like microbial metabolites, microbe–microbe interactions, diet, host physiology, immune response, and host environment.

14.7.2.1 Types of Dysbiosis

There are three types of dysbiosis which are as follows:

Type 1: It is caused due to loss of good bacteria in gut.
Type 2: It is caused due to gain of harmful bacteria in the stomach.
Type 3: It is caused due to loss of overall gut microbiome diversity, i.e., loss of both good and bad bacteria in the stomach.

Gut microbiota is associated with and has become an emerging field of current research, through the direct and indirect ways of diverse biological processes. These various biological processes associated include oncogenic signaling, immune system function, hormonal and detoxification pathways, chronic inflammation, host cell proliferation, and death. The play between the host and its own microbes is important and are associated with and plays a key role in the progress of various cancer, particularly breast cancer. The gut microbiota has played a key role as an additional environmental risk factor in the development of breast cancer. Gut microbiota dysbiosis involves the use of two mechanisms in breast cancer development, the first one is estrogen-dependent and the second one is non-estrogen-dependent mechanisms leading to the development of several microbial-derived metabolites, effects on DNA, immune regulation, and effects on DNA. The gut microbiota enhances estrogen by influencing estrogen metabolism (Ruo et al. 2021). In a clinical trial carried out with women for knowing whether the gut microbiota causes breast cancer, it was concluded in the study that the microbial pattern of diseased women was different than that of healthy women (Plaza-Díaz et al. 2019).

14.7.3 Host–Gut Microbiome Interaction

There are some of the most frequent bacteria found in the taxa are Bacillus Acinetobacter and Enterobacteriaceae are more predominant. Some other studies found that the bacterial strain of Staphylococcus and Propionibacterium was present in the taxa part of the women's breast. The iris found with more than 30% of *Enterobacteriaceae* was present in comparison to other microbiota. It was concluded that the administration of some probiotics may help to decrease the level of Escherichia coli content in the body. The level of microbiota level in the GUT is

interrelated to the microbiome level of the breast. It may help to increase or develop a strong immune system in children. The metabolic process of microbiota affects directly to the microbiota level of the breast and the content of the diet responsible for the growth of pathogenic and non-pathogenic bacteria. Metabolic product of the food material supports the growth of microbiota in the other part of the body like the breast and lungs and liver (Wang et al. 2021). Food metabolism by microbiota may decrease the risk of cancer and may prevent the growth of the tumor. Microbiota of the breast is also metabolites of toxic substances like carcinogens and toxins released by the pathogenic bacteria in the breast. A low level of microbiota in the breast is unable to neutralize the carcinogen and toxic metabolites of the pathogenic bacteria, and it increases the risk of breast cancer. Cadaverine is responsible for the synthesis of anticancer metabolites. A low level of cadaverine was found during the initial stage of breast cancer confirming that the progression of the tumor is high in the absence of cadaverine. The microbiota metabolite can generate oxidative stress which reduces the risk of breast cancer progression. One of the metabolites, lithocholic acid minimizes the chances of tumor proliferation in the breast. Lithocholic acid induces the nuclear erythroid 2-related factor 2. GUT microbiota increases the production of mammalian lignans such as enterolactone and other enterolignans which increase the signaling pathway of the estrogen and decreases tumor proliferation (Yang et al. 2021).

14.7.4 Probiotic Therapies in Breast Cancer

Microbiota can reduce the risk of breast tumor development by minimizing tumor proliferation and increasing the cell cycle arrest. Hassan et al. 2016 studied the effect of life and killed Enterococcus faecalis and Staphylococcus hominis present in breast milk for antiproliferative action. The researcher found that both strains can increase the apoptosis level in the breast cells which reduces the risk of breast tumor development. Similarly, *Lactobacillus Reuteri* was also reported for neutralizing tumorigenesis by increasing the rate of apoptosis (Lakritz et al. 2014). Some of the microbiota from the lactobacillus family help to generate IL-10 levels in the blood which is very beneficial for tumor regression and growth inhibition. Urbaniak et al. 2014 studied 81 women including lactating and nonlactating women to determine the microbiota content in the milk and found a variety of non-pathogenic lactobacillus family microbiota. Their research also found that some bacteria from the exterior part reached the duct. Banerjee et al. 2021 studied the effect of some strains of bacteria on four subtypes of breast cancer.

14.8 Conclusion

Synbiotics have considerable potential for cancer prevention and therapy although more clinical evidence is required. Many scientists have emphasized the importance of conducting prospective longitudinal cohort studies to find out the correlation

between synbiotics and cancer risk reduction. The main problem with symbiotic treatment is that everyone's flora is unique, so the flora composition must be determined prior to treatment. As a result, this could fall under the category of expensive medication. However, pro-, pre-, and synbiotics have great potential as a new strategy in the field of cancer prevention and treatment.

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Synbiotics in Gastroesophageal Cancer

15

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15.1 Introduction

The advancement of sequencing technology has increased our understanding of the human stomach microbiome, which is now known to show a promising role in maintaining a self-sustained balance and that changes in microbial community composition can encourage the development of gastric disorders. The carcinogenic consequences of the stomach microbiome have recently gotten a lot of attention. The most frequent occurring is gastric cancer (GC) having a significant fatality rate worldwide. *Helicobacter pylori* infection is a well-known GC risk factor. Apart from bringing some novel technology and technique for the diagnosis and therapy of gastrointestinal (GI) cancers, certain other factors are becoming increasingly significant, such as maintaining health and preventing malignancies through the use of human nutrition enriched with probiotics and prebiotics. Probiotics are live bacteria that carry the host's health advantages, when taken in adequate amounts (Indian Council of Medical Research Task Force et al. 2011). The fundamental advantage of probiotics is that they assist the host in maintaining intestinal microbial balance, reducing pathogenic gastrointestinal microbes, improving bowel regularity, and restoring intestinal microbial balance with antibiotic-related diarrhea. Moreover, probiotics have been shown to have potential role in the prevention of cancer and its treatment via modulation in microbiota and immune system and reducing

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bacterial translocation, improving gut barrier activity, anti-inflammatory and anti-pathogenic function, and different impacts on tumor formation and metastasis in several studies (Servin 2004; Cotter et al. 2005). Probiotics and gastrointestinal neoplasms have primarily been studied in concern with colorectal cancer (CRC) and gastric cancer associated with Helicobacter pylori (*H. pylori*) (Russo et al. 2014; Rasouli et al. 2017; Khoder et al. 2016; Taremi et al. 2005; Sanders et al. 2013; Ghosh et al. 2019). In addition, chemotherapy commonly causes severe diarrhea and oral mucositis in cancer patients, which has an impact on their treatment. Probiotics, when taken orally, give a therapeutic option for overcoming these limits. These findings suggest that probiotics could be used as dietary supplements to protect against neoplastic predisposition by influencing the immune system of the host (Zhang et al. 2011; Zuccotti et al. 2008; De Preter et al. 2011; Kumar et al. 2010; Liong 2008; Ghosh et al. 2019).

Lactic acid bacteria (LAB) from the Lactobacillus and Bifidobacterium genera are found in the majority of probiotic products now on the market (Holzapfel et al. 2001). Most of the probiotic microbes are Gram-positive, with Lactobacillus and Bifidobacterium being the most occurring species utilized in the treatment of gastrointestinal diseases (Marco et al. 2006). Some Gram-negative bacteria, on the other hand, are employed as probiotics. The most well-known member of this category is Escherichia coli Nissle 1917 (EcN) (12, commonly called "Mutaflor,"which has been recently used in the treatment of chronic constipation and colitis in Germany (Mollenbrink and Bruckschen 1994; Schutz 1989). Streptococcus thermophilus and Lactococcus lactis are two of the major economically important LAB that also show a major role in dairy products.

The current descriptive review highlights the most recent information on probiotic effects and mechanisms in GI malignancies. In addition, we have given a comprehensive evaluation of the evidence from clinical research employing probiotics to prevent or cure GI malignancies.

15.2 Probiotics

Probiotics are living bacteria that have health benefits when consumed by the body. They are found in yogurt, some fermented foods, and also in dietary additives and cosmetics. Probiotics involve a broad category of microorganisms among which bacteria from the Lactobacillus and Bifidobacterium genera are the most occurring ones. Similarly, other bacteria and some yeasts such as Saccharomyces boulardii can be used in probiotics. Probiotics have a wide number of structures where each has some specific advantages. It can be illustrated by taking an example of Lactobacillus. If one type of Lactobacillus prevents an illness, it doesn't mean that some other type of Lactobacillus or any type of Bifidobacterium probiotics will also prevent the same illness. The first requirements of probiotic strains are the safety and functionality for human and animal health along with their technical fitness, in accordance with World Health Organization (WHO), Food and Agriculture Organization (FAO), and European Food Safety Authority (EFSA). For the safety of any strain, there

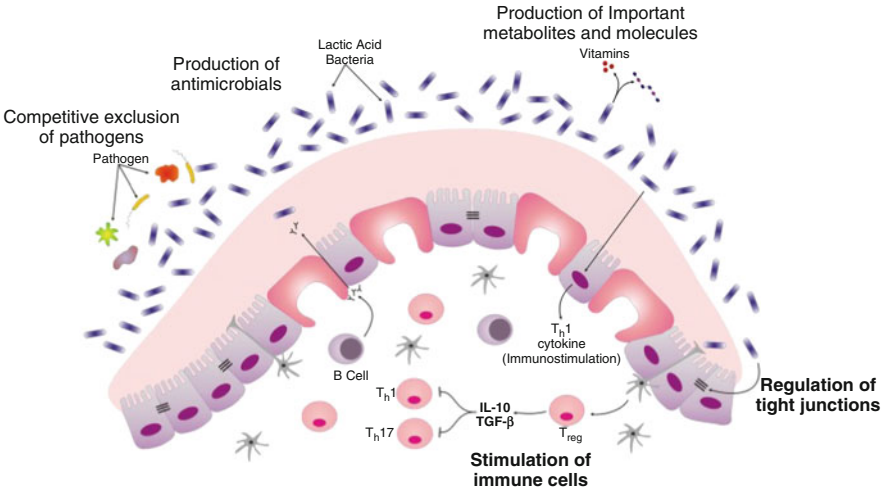

Fig. 15.1 Figure displaying different mechanism by which probiotic manifest their beneficial role in intestine. Figure reproduced with permission from Ghosh et al. (2019)

should be absence of connection between pathogenic cultures and the antibiotic resistance profile. The microorganisms which are employed as probiotics must satisfy the requirements of GRAS (Generally Regarded as Safe) and QPS (Qualified Presumption of Safety). Their survival in the gastrointestinal tract and its safety effects are determined by functional characteristics (Anadon et al. 2006; Gaggia et al. 2010). Because of the probiotic market's rapid growth, it's critical that probiotics survive and maintain their qualities throughout the storage and distribution process (Markowiak and Slizewska 2017) (Figs. 15.1, 15.2, and 15.3).

15.3 Prebiotics

Prebiotics, which are found naturally in some foods, can help our bodies create good bacteria. A prebiotic, in this case, is a substrate that is used selectively by host bacteria to provide health benefits. Prebiotics withstand hydrolytic behavior of the digestive enzymes in the stomach and small intestine, allowing them to transit to the colon. They're fermented here, which boosts the number of good bacteria in our gut. Many prebiotics are also classified as dietary fiber. Though not all fibers are prebiotics, insoluble fiber (the kind that adds bulk to the stool) is often poorly fermented by our gut bacteria and is therefore not considered a real prebiotic. Insulin, galactooligosaccharides, and fructooligosaccharides are the most studied prebiotics, which are also kinds of soluble fiber. Soluble fiber varies from insoluble fiber in which it forms a gel with water, trapping specific dietary components and slowing digestion. Insoluble fiber, on the other hand, adds bulk to the stool and aids in the faster passage of food through the stomach and intestine.

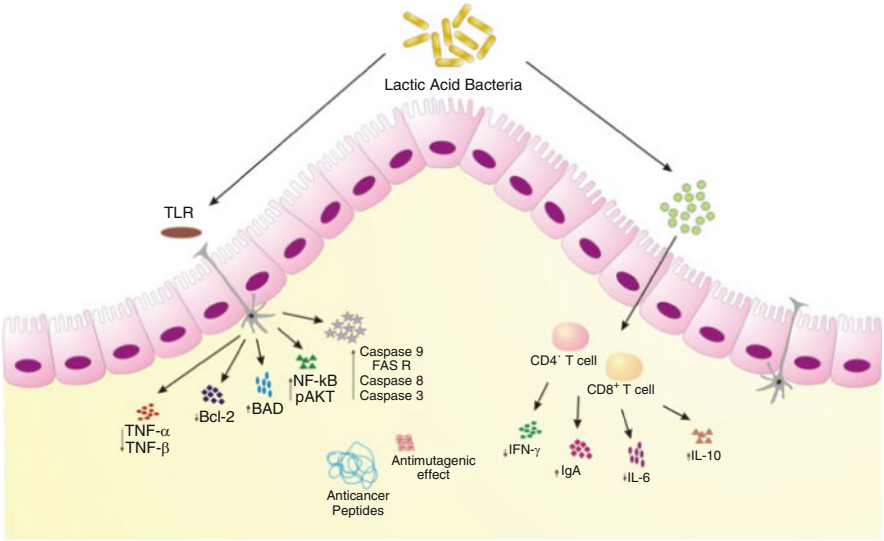

Fig. 15.2 Schematic diagram displaying different methods by which probiotic prevents intestinal cancer. Figure reproduced with permission from Ghosh et al. (2019)

15.4 Synbiotics

The term "synbiotics" was recently coined to describe a food that contains probiotics as well as prebiotics having a functional and health-promoting values (Schutz 1989). The main focus of synbiotics product is moving towards functional evidence such as infection resistance, antibacterial activity, and better immunological status (Schutz 1989). Though there have been a number of studies on biotic products that focus on a healthy colonic microbiota, there have been little studies on the actions of intestinal digesting enzymes. The impact of synbiotics which is a blend of probiotics and prebiotics, on the ecology of gut microbes and digestive enzyme behavior in rats, as well as the role of enteric feeding and the microenvironment in cancer must be studied thoroughly in upcoming days.

15.5 Anticancer Mechanism of Synbiotics

Probiotics have numerous anticancer properties as well as significant impact on the gut microbiota's quantitative and/or qualitative changes. One major cause for the development of GI cancer is the toxic and genotoxic bacterial metabolites from intestinal microbiota. These metabolites can cause mutations due to its binding to certain receptors of the cell surface and altering transductions of intracellular signals, which can lead to mutations. *Streptococcus bovis*, *Bactericides*, *Clostridia*, and *H. pylori* are among the bacteria that cause the development of cancer (Kasmi

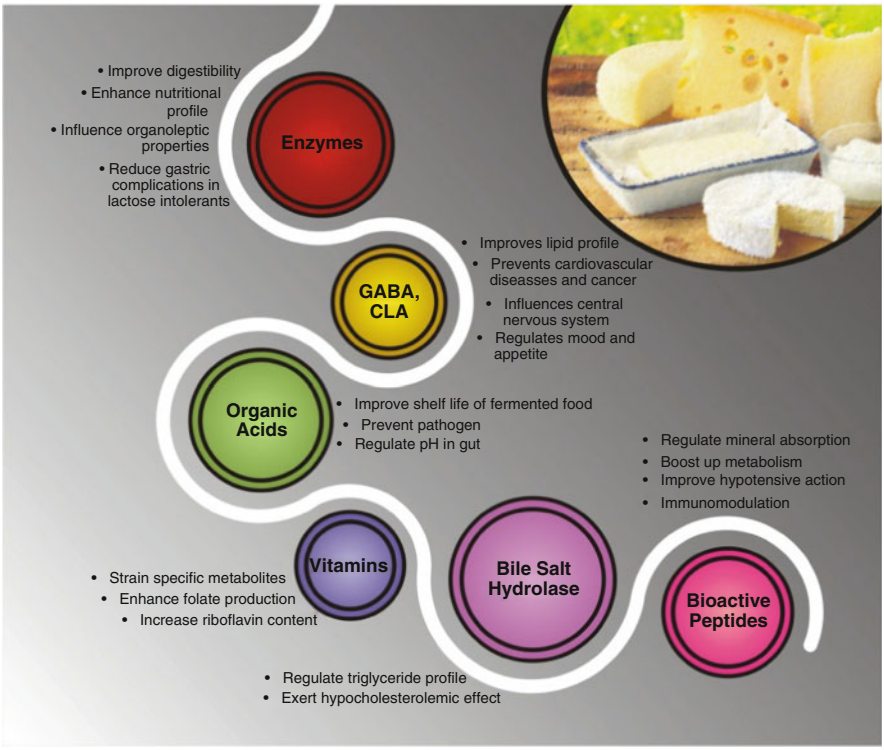

Fig. 15.3 Schematic diagram displaying health benefits of metabolites present in the fermented food. Figure reproduced with permission from Ghosh et al. (2019)

et al. 2011; Nakamura et al. 2002; Strofilas et al. 2012). As a result, the balance between "harmful" and "useful" bacteria has significance in the development of cancer. It has been observed that changing the number of microorganisms can affect carcinogen bioactivation and consequently cancer risk. Dietary components (prebiotics) are increasingly shown to have a substantial impact on this equilibrium. Furthermore, probiotics alter intestinal microbiological compositions, which has positive impact on the host by increasing intestinal barrier integrity, decreasing pathogen development, and reducing pro-carcinogenic chemical metabolism.

15.6 Role of Synbiotic in GI Cancer

The probiotic therapy can be used in the prevention and treatment of a variety of GI problems, such as irritable bowel syndrome (IBS), inflammatory bowel diseases (IBD), and the pathogenic bacterial or viral infection and antibiotic-related diarrhea, which has piqued the scientific community's attention (Zuccotti et al. 2008; De Preter et al. 2011). Probiotics have also been shown to protect against cancer in

epidemiological studies (Kumar et al. 2010). Probiotics have been shown to have anti-proliferative effects in the cancers of GI tract, with colonic as well as gastric cancer cells being the most widely examined (Liong 2008; Rafter 2004). Several research on the health impacts of fermented milk by using *Lactobacillus casei* and *Lactobacillus acidophilus* have been conducted, and the obtained results show that these probiotics have a good influence on tumor cell death (Lee et al. 2004; Baldwin et al. 2010). Previous research has shown that *L. rhamnosus* GG strain has anti-proliferative properties in both colon and gastric cancer cells of human (Russo et al. 2007; Orlando et al. 2009; Orlando et al. 2012), and there is an another probiotic product called *Bifidobacterium adolescentis* SPM0212 inhibited the proliferation of three human colon cancer cell lines: HT-29, SW 480, and Caco-2 (Kim et al. 2008). *Bacillus polyfermenticus* (Ma et al. 2010), *L. acidophilus* 606 (Kim et al. 2010), LGG/Bb12 (Borowicki et al. 2011), and *LGG/Bifidobacterium animalis subsp*. lactis were among the probiotic products or strains that showed anticancer activity for cancer cells of human colon (Stein et al. 2012). Cousin et al. also found that fermented milk containing *Propionibacterium freudenreichii* increased the cytotoxicity of camptothecin, a stomach cancer chemotherapy drug (Cousin et al. 2012). With the emergence of *H. pylori*-resistant strains, the efficacy of *H. pylori* eradication regimens involving two antibiotics (clarithromycin plus amoxicillin or metronidazole) and a proton pump inhibitor (PPI) has decreased in recent years. According to a recent meta-analysis, supplementing antibiotic therapy with probiotics is particularly effective in eradicating *H. pylori* (Tong et al. 2007; Losurdo et al. 2018; Zhu et al. 2014).

We further reviewed the findings of a research where clinical trials on the effect of probiotics and antibiotic combination in the eradication of *H. pylori* colonization. Probiotic addition during antibiotic treatment for *H. pylori* eradication reduces undesirable adverse effects which results in higher compliance and in some circumstances, enhanced eradication rates, according to the findings of these studies. Furthermore, after effective eradication, a stomach tumor that was stimulating lymphoid tissue development vanished (Gisbert and Calvet 2011; Kokkola et al. 1996). A postulated reason for probiotic treatment is due to the presence of microorganisms in the stomach though they remain there for a short period of time, thereby increasing the overall immune response and reducing the inflammation effect on the gastric mucosa of host cells due to *H. pylori* (Du et al. 2012).

Among frequently occurring disease in the world, colorectal cancer (CRC) is listed as third most frequent disease with over one million new cases diagnosed each year and over 500,000 people dying from it (Bhandari et al. 2017). Taking probiotics has been found to be a supportive and protective strategy for the maintenance of a healthy gut flora while also lowers the risk associated with colon cancer (So et al. 2017). Despite numerous in vivo and in vitro investigations in animal models and human cancer cell lines, few randomized placebo-controlled trials (RCTs) have reported on the efficacy of probiotics to prevent and inhibit in carcinogenesis the intestine (Hatakka et al. 2008; Worthley et al. 2009; Ohara et al. 2010).

To prevent intestinal malignancies is not only the benefit of probiotics but can also help patients who are having colon cancer surgery avoid symptoms and consequences.

Unlike several studies on CRC and GC, some studies have suggested a role for probiotics to prevent and treat other GI cancers of pancreas and liver.

Pancreatic cancer is the world's 12th most prevalent malignancy, with 338,000 new cases per year, and the 7th most common cause of death, with 331,000 fatalities per year; however, the cause is still unclear (Ferlay et al. 2017; Pourhoseingholi et al. 2017; Javanmaed et al. 2018). Previous research suggests that probiotics play a multidimensional role to prevent pancreatic cancer by modifying pancreatitis as well as other associated risk factors such diabetes, pancreatic necrosis, inflammation, and obesity (Olah et al. 2007; Olah et al. 2002; Besselink et al. 2008). Probiotics had no significant influence on the clinical benefits of patients with SAP, according to the results of a meta-analysis of six clinical trials (Gou et al. 2014). Because of only few trials and their heterogeneity, the existing data are insufficient to conclude the impacts of probiotics in pancreas and colon-associated cancer.

15.7 Conclusion

Various studies till this date have supported that synbiotics are supplementary diet-based strategies and has positive effect to prevent or treat GI cancer. In addition, synbiotics have proven to tackle cancer through several mechanisms like anti-carcinogenic effect, activation of the host immune system, alteration of tumor gene expression, and inhibition of proliferation of bacteria that changes pro-carcinogens to carcinogens. However, detailed clinical trials sufficiently proving advantageous role of synbiotics on GI cancer, mode of treatment and information on aftermath treatment are still insufficient. Hence in upcoming future additional evidence-based trial and thorough analysis of synbiotic in increasing the survival of patients with GI cancer must be performed.

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Role of Probiotics and Synbiotics in Mitigating Alcohol-Induced Liver Damage

16

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16.1 Introduction

Globally, in many societies and households alcohol drinking has become a part of daily life as a result of modernization and changing eating habits. Long-term alcohol consumption was the leading cause of liver failure and death related to liver dysfunction (Fuenzalida et al. 2021). Physiologically, long-term alcohol consumption can cause hepatic injury, which can lead to carcinogenesis and liver damage.

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"According to the Who statistics, the annual mortality toll from hepatic cirrhosis was 14 lakhs in 2010, making it the ninth largest cause of death in Western countries attributable to drug-induced damage" (Saleem et al. 2010). Alcoholic liver disease (ALD) is one of the leading causes of morbidity among alcohol use disorder (AUD) (Fuenzalida et al. 2021). On a global scale, ALD is the reason for 4.6% of all disability-adjusted life-years and 3.8% of all mortality-adjusted life-years, respectively (Lam et al. 2016). It is also the second most common reason for liver transplantation, affecting around 3.3 million patients every year (Mandayam et al. 2004; Lam et al. 2016; Dinis-Oliveira et al. 2015; Rehm et al. 2009).

16.2 Sequelae of Alcohol-Induced Pathogenesis

Globally, ALD is a common cause of cirrhosis, with substantial morbidity and death. The complicated interplay between the many metabolic intermediates of alcohol is thought to be responsible for its pathophysiology. Genetic and environmental variables, the immunological response, and the gut-liver axis connection are involved in triggering ALD (Fuenzalida et al. 2021). Chronic drinking alters the gut microbiota and consequentially causes liver dysfunction by affecting the barrier function in intestine and triggers free radical generation and inflammation. Alcohol metabolites such as acetaldehyde, malondialdehyde (MDA), which is produced as a by-product of lipid peroxidation and protein adducts are hepatotoxins that can cause severe damage by exacerbating systemic inflammation (Fuenzalida et al. 2021). Ethanol crosses the blood–brain barrier (BBB), triggers oxidative stress and inflammation, and culminates in damage irreversible changes in central nervous system (CNS) structures and brain functions (Fuenzalida et al. 2021).

16.3 Biochemical Metabolism of Alcohol

Biochemically, alcohol dehydrogenase (ADH) converts alcohol enzymatically through the oxidation process, resulting in ALD. As a result (Lieber 2004; Thurman et al. 1999; Zakhari 2006), acetaldehyde and acetate are produced. Acetaldehyde forms DNA and protein adducts and alters the structure and cell function (Lieber 1994; Mandayam et al. 2004; Zakhari 2006; Lam et al. 2016). The released acetaldehyde is harmful to mitochondria and their organelles, aggravating the oxidative stress (Lieber 2004). Microvesicular steatosis, nonalcoholic steatohepatitis (NASH), and cytolytic hepatitis are all linked events that damage mitochondrial DNA and its functioning (Jaeschke et al. 2002). The redox cellular state is altered and the generated reactive oxygen species (ROS) activate transcription factors of the genes involved in lipid production (Jaeschke et al. 2002). The most important are sterol regulatory element-binding proteins (SREBPs) and peroxisome proliferator-activated receptors (PPARs) (Ansari et al. 2016). In both alcoholic and non-alcoholic steatohepatitis, an increase in lipogenic transcription factors SREBPs and PPARs activates fatty liver formation (Ansari et al. 2016). Furthermore, the

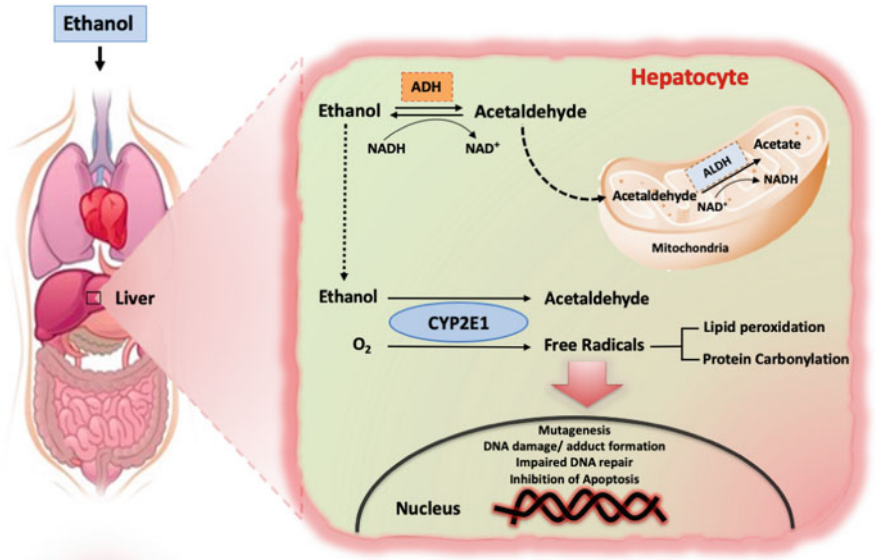

Fig. 16.1 Ethanol is converted to acetaldehyde by alcohol dehydrogenase (ADH) and, subsequently to acetate by aldehyde dehydrogenase (ALDH). Acetate is conjugated to coenzyme A and the resulting acetyl-CoA can be metabolized in the Krebs cycle, or utilized for the synthesis of fatty acids. In addition, a small fraction of ethanol is metabolized by cytochrome P450 2E1 (CYP2E1). The breakdown of alcohol triggers generation of free radicals which along with acetaldehyde leads to Mutagenesis, DNA damage/ adduct formation, Impaired DNA repair, Inhibition of Apoptosis all of which contribute to carcinogenesis.

acetaldehyde produced reacts with the carboxyl-terminal pro-peptide of procollagen and triggers collagen synthesis in hepatic stellate cells, (Lieber 2004). The mechanism is represented in Fig. 16.1.

16.4 Alcohol and Free Radical Generation

Chronic ethanol use causes free radicals to form, as well as increased hepatic oxygen demand and pathogenic alterations (Rehm et al. 2009; Dinis-Oliveira et al. 2015; Lam et al. 2016). Evidence also suggests that, alcohol metabolism also affects immune system functioning and lipid metabolism and the resulting hyper-lipid production complicates the underlying pathogenesis (Thurman et al. 1999; Lieber 2004; Lam et al. 2016). The simultaneous rise in ROS production activates the nuclear factors erythroid-2-related factor-2 (Nrf2) and hypoxia-inducible factor (HIF), making hepatocytes resistant to ethanol's harmful effects (Ansari et al. 2016). Inflammation is triggered and exacerbated by oxidative stress. Subsequently, these changes aggravate pathophysiology by increasing the synthesis of tumor necrosis factor-alpha, a proinflammatory cytokine in the liver's Kupffer cells (Lieber 2004).

Studies have also shown that cytochrome P450 system (CYP2E1) of liver microsomes is another metabolic pathway involved in alcohol poisoning and results in toxic end products (Lieber 1994; Lieber 2004; Zakhari 2006). The end products are cytotoxic than alcohol in damaging the liver damage. 4-hydroxynonenal, for example, is a peroxidative molecule that promotes collagen production and liver fibrosis (Lieber 2004). The acetaldehyde production, induction of CYP2E1, ROS generation, increased inflammatory responses, altered mitochondrial function, compromised antioxidant mechanisms; oxidative stress cumulatively causes death of hepatocytes by necrosis or apoptosis (Lieber 2004; Zakhari 2006).

16.5 Pathological Classification

The ALD spectrum is divided into three categories from a pathological standpoint. Fatty liver/hepatic steatosis is the first group. It refers to the fat buildup in the hepatic system of alcoholics. The second category includes alcoholic hepatitis, which causes inflammation to destroy liver cells, as well as alcoholic cirrhosis. Hepatic cirrhosis with extensive fibrosis and nodular regeneration causes sinusoidal intensification, increased vascular resistance, and deformed liver architecture. The destruction and consequent structural degradation results in severe functional damage, which can lead to other organs such as the brain, kidneys, and lungs malfunctioning (Fuenzalida et al. 2021). Although alcoholic cirrhosis and severe alcoholic hepatitis are associated with poor outcomes, a small number of people can recover with continued abstinence and supportive care (Fuenzalida et al. 2021). Finally, the severity of the disease may lead to liver cancer as a result of increased alcohol consumption (Lam et al. 2016). However, reports suggest only a small percentage of alcoholics progresses to hepatocellular carcinoma (HCC) and that cirrhosis, which is caused by excessive alcohol consumption, accounts for 40–90% of the 26,000 yearly deaths (DuFour et al. 1993).

16.6 Role of Gut Microbiota

The portal vein connects the gut with the liver in a bidirectional manner, both anatomically and physiologically. Bacteria, viruses, yeasts, and fungi are among the microorganisms that live in the gastrointestinal system, and their ratio always plays an important role in human physiology. This healthy habitat develops a long-term relationship with the host, resulting in a range of beneficial roles (Leclercq et al. 2019). Several studies have shown that both of these organs, as well as the microbiome and food, have a variety of consequences on liver disease and termed as “Gut-Liver Axis” (Albillos et al. 2020). Human intestine is home to nearly 500–1000 gut microbes, and a healthy balance between commensals and pathogenic microorganisms is maintained. The intestinal epithelium serves as a barrier between the microbiome and the liver and is a contact between the gut microbiota present in lumen and host immune cells (Albillos et al. 2020).

The usefulness of probiotics and synbiotics in preventing alcohol-induced hepatic damage and liver cancer has been extensively researched and affirmed to be beneficial to humans. Probiotics are live, non-pathogenic bacteria that can colonize the mucosa of the colon (Elzouki 2016). "In 2001, the Food and Agriculture Organization (FAO) in collaboration with World Health Organization (WHO) classified probiotics as live bacteria that, when given in sufficient proportions, promote the host's health" (Soccol et al. 2010). Probiotics are mentioned in ancient Hindu and Biblical literature as being beneficial to human health. The most typical probiotics are lactic acid bacillus (LAB) or Bifidobacterium (Bifidobacterium) strains, which are normal components of the gut flora (Ehrstrom et al. 2010). They are facultative or anaerobic bacilli that are Gram-positive and non-spore-forming (Shalev 2002). Their natural sources are milk and other dairy products such as curd and yogurt and Nobel laureate Illya Ilyich Metchnikoff linked the benefits of human health with yogurt intake to the microorganisms (Mackowiak 2013). Additionally, Tissier found that infants who were nursed had higher levels of Bifidobacterium-producing microorganisms in their stomachs, and that this helped to maintain healthy intestinal flora and prevent infections (Mackowiak 2013).

With concerted efforts scientists were eventually able to discover the impacts of probiotics on the metabolic, trophic, and protective effects on the human body after significant investigation for decades. The metabolic effects are ascribed to the digestion of non-digestible dietary lipids, endogenous mucus, nutrient absorption, and energy conservation. Trophic effects include epithelial cell proliferation control, homeostasis, and immune system regulation. The actions against pathogens and the improvement of barrier functions are the protective effects.

Gibson defined "a probiotic in 1995 as a non-digestible food item that benefits the host by selectively boosting the development and/or activity of one or a small number of bacteria in the colon, and thus improves host health" (Gibson et al. 2004). Additionally in 2016, an expert panel of the International Scientific Association for Probiotics and Prebiotics (ISAPP) updated the previous definition to the current form, which is a "substrate that is selectively used by host bacteria giving health benefit" (Gibson et al. 2017). It refers to a variety of dietary carbohydrate compounds, such as indigestible polysaccharides, oligosaccharides, galacto-oligosaccharides (GOS), or fructo-oligosaccharides (FOS), fructan (e.g., inulin) that selectively boost the colon's natural commensal microbiota and provide health advantages (Gibson et al. 2017).

Terminologically, the association of probiotics and prebiotics is termed "Symbiotic" and is useful to humans. This word was recently modified in 2019 by the ISAPP as "a mixture comprising living microorganisms and substrate (s) selectively utilized by host microorganisms that confers a health benefit on the host" (Swanson et al. 2020). Synbiotics are employed as nutritional and medicinal supplements as they have synergism that includes prebiotic selectivity for probiotic metabolism, ensuring bacterial survival and development in GI tract (Swanson et al. 2020; Gibson et al. 2004). In the recent past, targeted therapy for the gut microbiota is becoming more popular as a way to protect the body from the effects of alcohol-induced damage (Lu and Wang 2021). The colonic microbiome of patients with

hepatic diseases differs from that of normally healthy people. Previously, practitioners employed non-absorbable disaccharides to change the gut microbial environment to treat liver illnesses, such as hepatic encephalopathy, where lactulose was used to reduce the colonic pH and enhance ammonia excretion. Selective gut decontamination was a name used to describe this process. Prebiotics, probiotics, and synbiotics are three current approaches being investigated. With regard to the microbes, it is important that it can withstand the stomach's acidity and the bile's alkaline pH. Recently, a slew of commercially available versions of the aforementioned have flooded the market. They should, however, only be utilized in circumstances where unequivocal benefits can be demonstrated. Prebiotics and synbiotics have been found to have a favorable effect and can be utilized as an alternative therapy for ethanol-induced liver damage and cancer, according to recent research.

16.7 Probiotic Organisms

Lactobacilli are facultative anaerobes that can be found in the mouth, stomach, intestine, and even adult vaginal flora. During reproductive age, the glycogen in the epithelia of adult vagina ferments to lactic acid, lowering the pH to an acidic level, which protects against infections. In addition to biotin, vitamin B12, and vitamin K, lactobacilli in the colon synthesize a few minerals (Baati et al. 2000). Lactobacilli produce lactic acid from lactose and other sugars and vital for making cheese and other dairy products (Shalev 2002). They make antimicrobial chemicals like hydrogen peroxide, which inhibit disease growth, and they live in symbiosis with pathogens (Gänzle 2015). They also make biosurfactants, which prevent adhesion and stimulate macrophages, leukocytes, cytokines, and the immune system (Gudiña et al. 2011). Lactate is produced by the homofermentative process of glycolysis of hexoses, and hexoses are metabolized by the heterofermentative process of phosphoketolase to lactate, carbon dioxide, and ethanol or acetic acid. *Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus gasseri, Lactobacillus casei, Levilactobacillus brevis, Limosilactobacillus reuteri*, and *Limosilactobacillus fermentum* are among the lactobacilli strains known as lactic acid bacteria (LAB).

16.7.1 *Lactobacillus acidophilus*

Lactobacillus acidophilus is a Gram-positive bacillus found in gut of both humans and animals. They use a homofermentative technique to ferment sugar (Baati et al. 2000). It contains probiotics and is used to make yogurt, together with Streptococcus thermophiles. These bacteria reduce blood cholesterol while raising feces cholesterol when fed to pigs, which have a gut similar to humans. *Clostridium perfringens,* S*taphylococcus aureus, Escherichia coli*, and *Salmonella typhimurium* are also prevented from growing (Gilliland and Speck 1977). They also lessen the effects

of *Streptococcus mutans*-caused dental plague. They protect against infection by yeast such as *Candida albicans* because they are part of a healthy vaginal flora. It may cause bacteremia when given to those who are immuno-compromised/suppressed, have central venous catheters, or are preterm newborns (Durchschein et al. 2016).

16.7.2 *Lactobacillus plantarum* or *Lactiplantibacillus plantarum*

Lactobacillus plantarum, commonly known as *Lactiplantibacillus plantarum*, is a homofermentative, aerotolerant bacteria that produces both lactic acid isomers. In the presence of heme and menaquinone, they produce cytochrome and use oxygen for respiration (Pedersen et al. 2012). Insects and vertebrates have these in their intestines. They thrive in a pH range of 3.4–8.8. Dairy products and fermented vegetables such as brined olives (Randazzo et al. 2010), sausages, and stock fish contain them. It contains antioxidant properties and aids in preserving intestinal permeability. It helps with inflammatory bowel syndrome (IBS) because it inhibits the usual gas-forming bacteria in the intestine (Bixquert Jimenez 2009) and produces antibacterial compound against other bacteria.

16.7.3 *Lactobacillus gasseri*

Lactobacillus gasseri is also found in the natural flora of the vaginal cavity. Lactocillin and bacteriocin gassericin A are produced. When *Lactobacillus gasseri* was given to rats that had been provided with acute dose of alcohol, the serum alcohol and acetaldehyde levels were less when compared to controls (Lim et al. 2021). When the strains of *L. gasseri* isolated from the feces of healthy newborn child were investigated for the basic adhesion and aggregation properties, both the viable a non-viable forms autoaggregated and co-aggregated with the pathogenic *Cronobacter sakazakii* (ATCC 29544) and *Clostridium difficile* (1296). A clinical trial performed to evaluate the effects of *L. gasseri* in healthy individuals vaccinated with trivalent Influenza (A/H1N1 and B) vaccine showed that the protective antibody titer was increased in probiotic administered group (Nishihira et al. 2016). Thus, this strain increases the immunity in healthy individuals.

16.7.4 *Lactobacillus casei*

Lactobacillus casei is an anaerobic LAB that can be found in the human reproductive and gastrointestinal tracts. It is utilized in the fermentation of dairy products and has probiotic effects. It produces amylase, a carbohydrate digesting enzyme. They have an inhibitory effect on *Helicobacter pylori* in vivo. When compared to a control group in a clinical trial, they reduced infection by *Clostridium difficile* and diarrhea due to antibiotic administration (McFarland 2009). They've been utilized to reduce

the amount of chemicals that cause flatulence caused by natural bean fermentation (Takeda and Okumura 2007).

16.7.5 *Levilactobacillus brevis*

Levilactobacillus brevis is a heterofermentative LAB that can be discovered in the human intestine and vagina (Zheng et al. 2020). They may live in anaerobic conditions. It can be present in fermented foods such as pickles, and it can also cause beer to deteriorate. It makes dextran and kefiran polysaccharides, as well as biogenic amines including tyramine and phenylethylamine (Pidoux 1989). Due to the high quantity of hydrogen peroxide produced, its presence in the vaginal area inhibits infection with yeast and Trichomonas species (Eschenbach et al. 1989). They can't turn milk into yogurt, but when combined with milk in geriatric patients, they help to improve cellular immunity.

16.7.6 *Limosilactobacillus reuteri*

Limosilactobacillus reuteri is found in the intestine and feces of people, as well as livestock such as chickens, sheep, and pigs. Reuterin, reutericin, and reutericyclin are bacteriocins produced by them. Reuterin is a new broad-spectrum antibacterial material made from glycerol fermentation (Talarico et al. 1988) and inhibits a range of bacteria, fungi, and protozoa (Talarico and Dobrogosz 1989), as well as other unicellular parasites. In children, it has been utilized as an adjuvant therapy for *H. pylori*. They have a cidal effect on *Streptococcus mutans* in the oral cavity. They have conferred high levels of resistance to *Salmonella typhimurium, Escherichia coli* in chicken, and *Cryptosporidium parvum* in mice and pigs in animal models such as mice (Casas and Dobrogosz 2000).

16.7.7 *Limosilactobacillus fermentum*

Limosilactobacillus fermentum is a heterofermentative LAB found in vertebrate intestines. This bacterium has inherent antibiotic resistance and is a possible carrier of resistance genes to humans from animals or the environment (Klein 2011). It has excellent bile tolerance, survive at pH 3 and is ideal for use as a probiotic (Pan et al. 2011). They can also lower cholesterol by speeding up cholesterol metabolism and increasing the demand for bile salt, which is generated from cholesterol (Pan et al. 2011). They reduce pathogenic *Salmonella* spp., *Shigella* spp. in the intestine, and UTI caused by *E.coli* and *Staphylococcus* spp. as a probiotic in dairy products (Mikelsaar and Zilmer 2009).

16.7.8 *Bifidobacterium* Species

Bifidobacterium species are anaerobic bacteria that are Gram positive, nonmotile, and Y-shaped. They're found in mammals' lower female genital tracts and gastrointestinal tracts. In adults and children, they are classed as plant-derived fructooligosaccharides or dairy-derived galactooligosaccharides, respectively, based on metabolism (Mayo 2010). Because it maintains intestinal microbe homeostasis, inhibits pathogens and harmful bacteria, modulates local and systemic immune responses, produces vitamins, and produces bioactive molecules from dietary substances, *Bifidobacterium* are ideal probiotics in managing ulcerative colitis (Ghouri et al. 2014). They are known as scavengers of the intestines. They are engaged in the carbon and energy metabolism of complex oligosaccharides. They use glucosaminidases and mannosidases to ferment galactomannan-rich natural gum, which ferments glucosamine and mannose, respectively. *Bifidobacterium longum* and *Bifidobacterium breve* are two common probiotic bacteria. *Bifidobacterium longum* helps to regulate the immune system, reducing the duration and intensity of the common cold. *Bifidobacterium breve*, a bacteria obtained from human newborn feces, has been found to help with ulcerative colitis, *Helicobacter pylori* treatment, and irritable bowel syndrome pain, bloating, and constipation. With *B. breve*, pre-obese people were able to avoid or reverse obesity (Mayo 2010; Ghouri et al. 2014).

16.7.9 Probiotics in Mitigating Alcohol-Induced Liver Damage

LAB strains such as *Limosilactobacillus fermentum*, *Limosilactobacillus reuteri*, and *Levilactobacillus brevis* have been demonstrated to protect ethanol-induced HepG2 cells in several experiments. By modulating CYP2E1, antioxidant enzymes (SOD, GPX, and CAT), lipid synthesis factors (SREBP1C and FAS), and lipid oxidation factors (PPAR, ACO, and CPT-1), these strains protected the liver from alcohol-induced hepatic damage (Liu et al. 2021). Ethanol-induced damage and detrimental post-translational changes of heat shock protein (Hsp60) chaperones were significantly reduced in *L. fermentum*. Following probiotic therapy, steatosis, iNOS levels, and Hsp60 levels all decreased (Barone et al. 2016). *L. plantarum* HFY09 (LP-HFY09) showed a decrease in various hepatic parameters like serum triglyceride (TG), total cholesterol (TC), SGOT, SGPT, hyaluronidase (HAase), and precollagen III (PC III) and a rise in liver alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase in mice with ALDH. *Lactobacillus plantarum* HFY09 helped to reduce inflammation by increasing interleukin 10 (IL-10) levels and lowering proinflammatory factors [IL-6, IL-1, and tumor necrosis factor (TNF)].

Lactobacillus plantarum HFY09 increased hepatic superoxide dismutase (SOD) and glutathione (GSH) levels while lowering liver malondialdehyde levels (MDA).

When compared to commercial *Lactobacillus delbrueckii* preparations, it showed improved modulation of hepatoprotective activities. The upregulation of peroxisome proliferator activated receptors, SOD1, SOD2, glutathione peroxidase (GSH-Px), nicotinamide adenine dinucleotide phosphate (NADPH), and catalase (CAT), as well as the downregulation of cyclooxygenase-1 (COX1), c-Jun N-terminal kinase (JNK), and additional ERK. For persons who consume alcohol often, the administration of LP-HFY09 could be a potential intervention (Gan et al. 2021).

Toll-like receptors (TLR) are found in immune cells and hepatocytes, and they recognize bacterial components that go from the stomach to the portal vein. In the absence of microbial components, ethanol activates TLR, resulting in an increase in proinflammatory cytokine production. According to research, *L. casei* MYL01 reduced ethanol-induced proinflammatory responses and increased TLR tolerance to ethanol activation. This was attributable to increased IL-10 synthesis, toll interacting protein (TOLLIP), and suppressor of cytokine signaling (SOCS)1 and SOCS3 expression via TLR1, TLR2, TLR6, and TLR9 activation, which cross-regulated ethanol-TLR4-nuclear factor B signaling events. All of these substances suppressed the pro-inflammatory response and boosted hepatocyte defenses against ethanol-induced injury (Chiu et al. 2014). Additionally, extract derived from green tea (*Camellia sinensis*) and fermented with *Lactobacilli fermentum* strain OCS19 mitigated acute alcohol-induced liver damage in both HepG2 hepatic cell line and in mouse model of study (Park et al. 2012). Administering the *L. fermentum*-fermented green tea extract (FGTE) increased the activity of hepatic alcohol dehydrogenase (ADH) and its mRNA expression indicating that combining green tea extract with *L. fermentum* fermentation reduces the risk of ethanol-induced liver damage (Park et al. 2012).

16.8 Conclusions

The current review highlights the use of probiotics by inhibiting and mitigating the ethanol-induced hepatic damage (Fig. 16.2). Multiple pathways are triggered to mitigate these effects and the principal are the antioxidant, anti-inflammatory and the protective events in the gut-liver axis. Future endeavors should be focused towards understanding the usefulness of probiotics in well-planned randomized clinical trials as the outcome of these will be useful for both fraternity and the society.

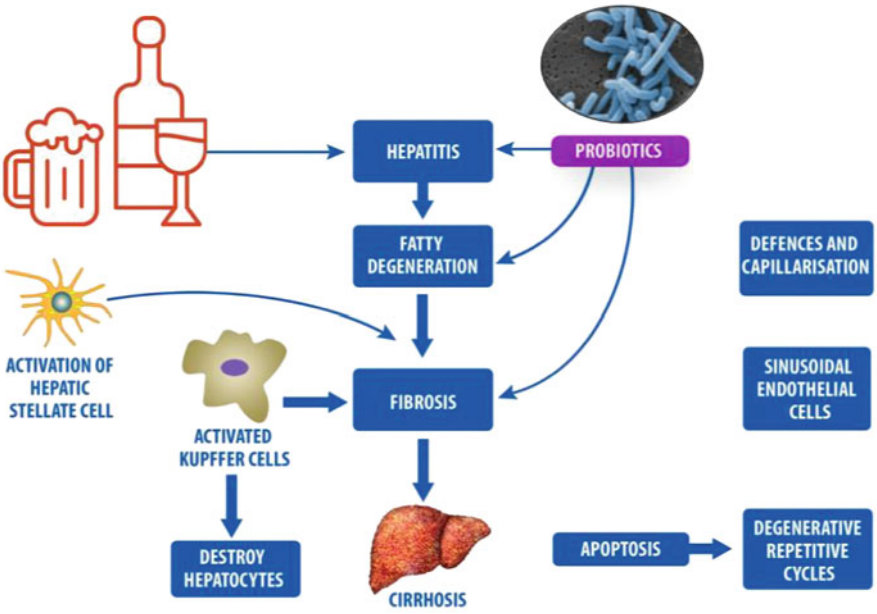

Fig. 16.2 Mechanism/s by which probiotics mediate the hepatoprotective effects against the alcohol induced liver damage

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Understanding the Role of Synbiotics in Prevention and Management of Cervical Cancer

17

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17.1 Introduction

The rapid increases of oncological diseases are now the main global burden which is multifactorial and caused due to genetic as well as environmental factors, such as dietary and lifestyle habits. Some environmental factors significantly change the host gut microbial community, which leads to induce changes in host physiology and contributes to the development of numerous diseases such as cancer (Marta et al. 2020). In current scenario, for the management of various diseases including cancers, synbiotics are used.

17.2 Synbiotics: An Overview

The concept of synbiotics was introduced firstly by Glenn R. Gibson and Marcel B. Roberfroid in 1995. They defined it as "a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare" (Gibson and Roberfroid 1995). However, along with time, as the definitions of prebiotics and probiotics were changed, the definition of synbiotics has also been updated. In May 2019, the panel gathered by the International Scientific Association for Probiotics and Prebiotics (ISAPP) defined synbiotics as "a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health

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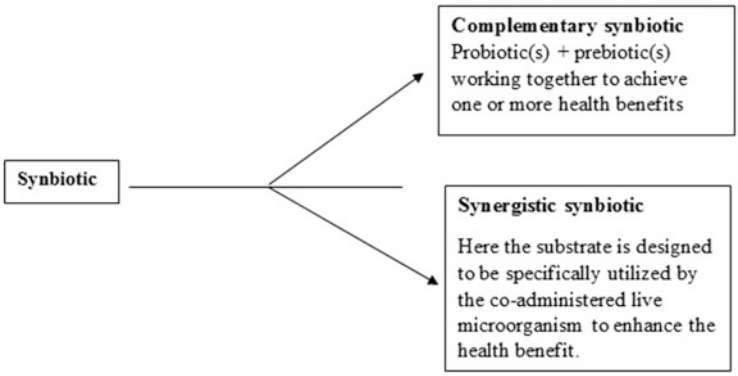

Fig. 17.1 Classification of synbiotics

benefit on the host." They also clarified the two types of synbiotics (Fig. 17.1). Complementary synbiotics are defined as a combination of prebiotics and probiotics having heath benefit(s) but functioning independently. In synergistic synbiotics, the substrate is designed, so that the co-administered microorganisms can selectively utilize it (Swanson et al. 2020).

Several positive effects of synbiotics on human nutrition and health have been reported. It was reported that activation of digestive enzymes like sucrase, lactase, isomaltase along with the reduction of coliform bacteria were observed after the application of synbiotics and they significantly increased the number of probiotic bacteria in the fecal sample (Yang et al. 2005; Yadav et al. 2022). Application of synbiotics also reported to reduce the risk of various metabolic disorders like type 2 diabetes, cardiovascular disease, and cancer (Cicero et al. 2021; Yadav et al. 2022). Several therapeutic potentials of synbiotics were also observed. A positive effect of synbiotics on diseases like sepsis in early infancy, hepatic conditions, obesity, type 2 diabetes, insulin resistance, irritable bowel syndrome, and cancer were also observed (Yadav et al. 2022). Ongoing clinical trials of synbiotics on Sars-Cov2 infected patients is nowadays also suggested a reduction of its gastrointestinal symptoms (Xavier-Santos et al. 2022).

17.3 Effects of Synbiotics on Cancer

One of the major probiotic bacteria such as *Lactobacillus* sp. can utilize the prebiotics for their own growth in synbiotic food products (Yadav et al. 2022). Interestingly, this combination of pro- and prebiotics has a greater effect than individual pre- or probiotic administration (Fotiadis 2008). Symbiotics are not only modifying the host microbiome, but they can also act as antimutagens. These synbiotics play a vital role in scavenging and eliminating carcinogens. Growing evidence suggested that the symbiotics modulate the adverse effects of chemotherapy in cancer patients, thus it is widely used for the treatment of cancer (Qiu et al.

2019; Tian et al. 2019). Many anticancer drugs are designed for the treatment of malignancies and most of them are generally toxic for healthy cells with numerous side effects and some of which are life-threatening also. In past several years, chemotherapy and immunotherapy are used for cancer treatment. But there are many limitations in these types of the treatment procedure. It is reported that these anticancer therapies affect the microbiota profile in patients and induce high toxicity (Panebianco et al. 2018). Current studies also reveal that synbiotics have many beneficial effects on human health as well as they have a very limited side-effect profile. It is observed that cancer patients are often in a state of immunocompromised due to property of cancer cell itself or by the treatment regime. In recent years, many studies are oriented towards the administration of synbiotics as a principal therapy in regard to cancer with minimal side effects. By different mechanisms, synbiotics show their oncosuppressive effects by preventing of host cell proliferation, maintaining intestinal barrier function, and immunomodulation. There are some strong evidences which suggested that the human microbiota plays an important role in carcinogenesis. A large proportion of cancer patients usually consume antibiotics for their therapeutic perspective, but use of these antibiotics has a large impact on host–microbiome composition and function (Francino 2015). It was reported that antibiotic-treated patients had worse overall survival when compared with those patients treated with synbiotics. Thus, in comparison to negative manipulation of microbiota with antibiotics, synbiotics currently represent the alternative therapy towards the positive manipulation of host microbiome and thus now it is used for potential therapeutic treatment in cancer (Scott et al. 2018). A meta-analysis also confirms that symbiotics can minimize the adverse effects associated with surgery, chemotherapy, radiotherapy, and antibiotics (Marta et al. 2020). Another important role of synbiotics is to prevent the conversion of non-toxic pro-carcinogens to harmful carcinogens, resulting in reducing the carcinogenic effects (Marta et al. 2020). Some other evidences suggested that use of particular synbiotics results in reduced levels of chemotherapy- and radiotherapy-related diarrhea and post-surgery infectious complications.

17.4 Cervical Cancer: A Major Global Burden Among Women

According to GLOBOCAN, 2021, cervical cancer appeared to be the fourth most common cancer among women in the world. Oncogenic Human Papillomavirus (HPV) was identified as primary causal factor for cervical cancer. It is already known that in cervical cancer cases, HPV E6 and E7 oncoprotein interact with p53 and pRB tumor suppressor genes and suppress their expression for the development of cervical cancer. In recent years, multidisciplinary approaches are used for the treatment of cervical cancer. It was observed that symbiotic supplementation can reduce the adverse gastrointestinal side effects of various cancer patients including patients of cervical cancer (Jahanshahi et al. 2020). Now different studies tried to understand the molecular mechanistic pathways of synbiotics in the treatment of various cancers including cervical cancer. Studies on cervical cancer also suggested

that the presence and enrichment of some specific bacterial species may resist HPV infection in the cervix, and these beneficial bacterial communities also help to clear off the HPV infection and reduce the risk of the development of cervical cancer. Thus, in the future, for better and safer oncological treatment, synbiotics can be used, which provide a great opportunity as an alternative therapeutic strategy.

The use of these therapies, both chemo and radiation with surgery effectively abolish the growth of cancerous cells in cervix. But these therapies induce several short- and long-term effects on the patients and thereby lead to several side effects. The adverse side effects are pain, nausea, vomiting, and fatigue (Cho and Blaser 2012). The chemoradiotherapy are generally applied on patients with locally advanced cervical cancer which are restricted to the pelvis (Eifel 2006). Other patients who are treated with concurrent chemotherapy in addition to the radiotherapy have increased gastrointestinal side effects (Eifel 2006). This side effect of nausea and vomiting could lead to severe diarrhea and weight loss. In recent years, the understanding of overall importance of microbiome in our lives has increased, also its role in cancer. Disturbances in the vaginal microbiota composition may play an important role in cervical cancer pathogenesis. Therefore, microbiota-based therapy can serve as a better option for cervical cancer prevention and treatment (Nelson et al. 2015). The beneficial effects of synbiotics on cervical cancer therapy are reported by various studies, and application of these class of therapy can reduce the risk of gastrointestinal side effects by the conventional chemotherapeutic strategies.

17.5 Synbiotics as Therapeutic Strategy in Cervical Cancer

The two categories of synbiotics may help to understand the correlation between prebiotics and probiotics and ultimately the formulation of synbiotic products for the beneficial effect on cervical cancer and therapeutic application.

In synbiotics, probiotics serve as the major component. Probiotics are living microorganisms, which have health beneficial effects when consumed or applied to the system. It may contain diverse microorganisms. The most common one in probiotics are *Bifidobacterium* and *Lactobacillus* and some yeasts like *Saccharomyces boulardii,* etc. Probiotics have diverse characteristics which are summarized in Fig. 17.2 (Morelli and Capurso 2012; Han et al. 2021; Krishnamoorty et al. 2022).

It was already established that, probiotics can modulate cancers via induction of apoptosis, inhibition of mutagenic and kinase activity, downregulation of oncogenic expression, induction of autophagy, activation of tumor suppressors, and inhibition of metastasis (Kim et al. 2010; Motevaseli et al. 2017; Jahanshahi et al. 2020). Various studies demonstrated the effect of probiotics on cervical cancer therapy. Some studies on cervical cancer cell lines like HeLa, Caski, and SiHa reveals the effect of some probiotics which are summarized in Table 17.1.

Based on these in vitro studies, it was identified that probiotics have amazing abilities to prevent or regress cervical cancer by reduction of cellular proliferation, metastasis and inflammatory response, and induction of apoptosis. Not only that,

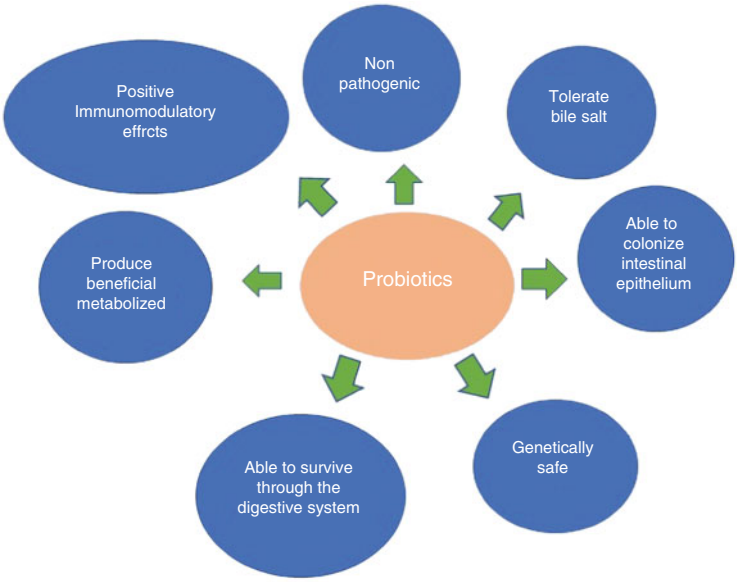

Fig. 17.2 Schematic diagram representing the characteristics of probiotics

Table 17.1 Effects of probiotics on cervical cancer cell lines

Probiotics	Cell line	Observation	Reference
L. jensenii, L. crispatus, and *L. gasseri*	Caski	Decrease of cell viability by modulation of HPV oncogenes and cell cycle	Jahanshahi et al. (2020), Wang et al. (2018)
L. plantarum	HeLa	Induce apoptosis and decrease cell proliferation	Jahanshahi et al. (2020), Nami et al. (2014)
L. casei and L. paracasei	HeLa	Augment apoptosis	Jahanshahi et al. (2020), Rajoka et al. (2018)
L. gasseri	HeLa	Reduce cellular proliferation and inflammatory response and induce apoptosis	Jahanshahi et al. (2020), Sungur et al. (2017)
L. crispatus and *L. rhamnosus*	HeLa	Reduce cell proliferation and metastasis	Jahanshahi et al. (2020), Nouri et al. (2016)
Bifidobacterium adolescentis SPM1005-A	SiHa	E6 and E7 oncogenes suppression	Cha et al. (2012), Jahanshahi et al. (2020)

probiotics with other chemotherapeutic drugs exert better results (Kim et al. 2015; Jahanshahi et al. 2020; Negi et al. 2020). Administration of probiotics confers prevention against gastrointestinal side effects caused by cervical cancer therapies

in combination with conventional anti-infective drugs. Some studies proposed that in reduction of incidence of diarrhea, the probiotics have a beneficial role (Liu et al. 2017; Jiang et al. 2021). A study identified that supplementation of probiotics reduces radiation-induced diarrhea among cervical cancer patients effectively (Linn et al. 2019). Other studies also reported modest reduction in incidence of diarrhea of cervical cancer patient undergoing chemoradiotherapy by using probiotic liquid yogurt (Giralt et al. 2008; Liu et al. 2017; Linn et al. 2019). The most common used probiotics are *Lactobacillus* and *Bifidobacteria* in these studies. Lactobacillus-based treatment can enhance p21 tumor suppressor expression in cervical cancer cell lines (Wang et al. 2018). *Lactobacillus plantarum* are cultured from vaginal secretions of young adult and adolescent women, and it exhibited probiotic and anticancer features in HeLa cervical cancer line (Nami et al. 2014). Another study also revealed that Lactobacillus strains that were isolated from human milk have remarkable antioxidant activity, resistance to low pH and high level of bile salts, antibiotic susceptibility, and probiotic characteristics (Rajoka et al. 2018). Exopolysaccharides of *L. gasseri* strains in lyophilized state induce apoptosis in HeLa cells in relation to Bax and Caspase3 upregulation (Sungur et al. 2017). *L. gasseri* also reduces TNF-α and increases IL-10, which leads to their anti-inflammatory impact on HPV-induced cervical cancer. Supernatants of *Lactobacillus crispatus* and *Lactobacillus rhamnosus* also reduced the expression of matrix metalloproteases like MMP2 and MMP9 along with CASP3 and eventually metastasis in HeLa cell line (Nouri et al. 2016). In terms of management of gastrointestinal symptoms in cervical cancer patients, it is reported that administering a probiotic with live *Bifidobacterium animalis* subsp. lactis BB-12 and *Lactobacillus acidophilus* LA-5 associated with reduced development of severe diarrhea after beam pelvic radiotherapy (Linn et al. 2019). In another study, a probiotic drink consisting of *Lactobacillus casei* was employed on cervical cancer patients who had undergone radiotherapy and cisplatin-mediated therapy, and this application is proven to be beneficial for improving stool consistency (Giralt et al. 2008). Microbiome also serves as a biomarker for diagnosis of cervical cancer. A study using *Lactobacillus rhamnosus* and *Lactobacillus reuteri* serves as a promising biomarker for detection of cervical malignancies (Perisic et al. 2011). A study on 228 stage IIIB cervical cancer patients, combination therapy with heat-killed *Lactobacillus casei* (LC9018) with radiotherapy significantly improved the response pattern of the patients (Okawa et al. 1993). It was also reported that LC9018 can be used as adjuvant and associated with longer disease-free survival among patients who had undergone radiotherapy alone. Another study also reported that the pessaries containing both cisplatin and probiotic biomass can be utilized as better therapeutic method for cervical cancer patients, and they are reported as good scavenger for free radicals (Negi et al. 2020).

Prebiotics serve as another major component in synbiotics. They are basically compounds in food, which can promote the proliferation or activity of beneficial microorganisms including bacteria and fungi. Normally, dietary prebiotics is nondigestible food ingredients that travel undigested through the upper part of the intestine and stimulate the activity and growth of beneficial microorganisms by acting as a substrate for them (Markowiak and Slizewska 2017). Cereals, vegetables,

Table 17.2 Most commonly used prebiotics, probiotics, and synbiotics for human

Human nutrition		
Prebiotics	Probiotics	Synbiotics (probiotics + prebiotics)
Fructooligosaccharides (FOS) Galactooligosaccharides (GOS) Xylooligosaccharides (XOS) Inulin Lactitol Lactulose Soy oligosaccharides TOS (Transgalactooligosaccharides) Lactosucrose	*Lactobacillus* genus bacteria like *L. jensenii, L. crispatus, L. plantarum, L. gasseri, L. casei, L. rhamnosus,* etc. *Bifidobacterium,* Some yeast like *Saccharomyces Boulardii, etc.* *Streptococcus sp.* *Enterococcus sp.*	Inulin+ *Lactobacillus* genus bacteria FOS+ *Bifidobacterium, Lactobacillus,* and *Streptococcus* genus bacteria FOS+ *Bifidobacterium, Lactobacillus, Enterococcus* genus bacteria Oligofructose+ *Bifidobacterium, Lactobacillus* genus bacteria + oligofructose Inulin+ *Bifidobacterium* and *Lactobacillus* genus bacteria

and fresh fruits serve as the good sources of prebiotics. Specifically, green vegetables, garlic, onion, tomatoes, artichokes, bananas, asparagus, berries, chicory, green vegetables, legumes, as well as oats, linseed, barley, and wheat are potential sources of prebiotics (Crittenden and Playne 2008; Markowiak and Slizewska 2017).

Some artificial prebiotics are also reported such as lactulose, maltooligosaccharides, galactooligosaccharides (GOS), and lactosaccharose. Fructans, like inulin and oligofructose, have an effective relationship with various types of probiotics (Markowiak and Slizewska 2017). Like probiotics, there are many reports regarding the beneficial effects of prebiotics on malignancy. Some in vitro studies on human colorectal cancer cell lines (L97 and HT29) demonstrated that inulin fractions on plasma supernatant reduced growth and promote apoptosis in human colorectal carcinoma cell lines (Munjal et al. 2009; Markowiak and Slizewska 2017). This study supports that prebiotic has an impact on cancer. Not only colorectal cancer, but some in vitro studies also observed that employment of inulin and oligofructose (dose 5–15%) exerts beneficial effect on breast cancer and resists metastases to the lungs (Markowiak and Slizewska 2017; Taper and Roberfroid 2002). Studies between prebiotics and cervical cancer are now unclear but previous reports of prebiotics on various types of cancer justified that there is a close relationship between cervical cancer and prebiotics.

It was already known that a synbiotic is a mixture of probiotics and prebiotics which significantly affects the host by improving the growth and activity of beneficial gut microbiota.

Table 17.2 represents the combination of some popularly used probiotics and prebiotics used as a synbiotics (Crittenden and Playne 2009; Olveira and Gonzalez-Molero 2016; Saez-Lara et al. 2016).

It was found that, the application of a symbiotic product containing blended probiotics *(Lactobacillus rhamnosus, Lactobacillus casei, Lactobacillus bulgaricus,*

Lactobacillus acidophilus, Bifidobacterium breve, Streptococcus thermophilus, Bifidobacterium longum) and fructooligosaccharides as prebiotic resulted in the downregulation of nuclear factor B and decreased expression of TNF-*a* (Markowiak and Slizewska 2017; Eslamparast et al. 2014). Findings demonstrated that patients who were given synbiotic containing Bifidobacterium lactis Bi-07 1 × 106 CFU/g biogel, 1 × 107 (CFU)/g biogel of *L. acidophilus* NCFM, and inulin reduced levels of fecal calprotectin and less incidence of intense vomiting in cervical cancer patients (De Loera Rodriguez et al. 2018). Thus, synbiotic supplementation may be beneficial for reducing gastrointestinal side effects of cervical cancer patients.

17.6 Influence of Synbiotics on HPV Infection in Cervical Cancer

It is established that use of synbiotics can reform and maintain a healthy balance of bacterial species. Also, it is seen that the use of oral probiotics has effectively treated gastrointestinal diseases such as irritable bowel syndrome, traveler's diarrhea, gastroenteritis, and others (Champer et al. 2018). Interestingly, it has been shown that synbiotics such as *Lactobacilli*-based treatment results in the downregulation of cyclin A, CDK2, and HPV oncogenes E6 and E7 (Wang et al. 2018; Yim and Park 2005). The first report of the use of *Lactobacillus rhamnosus* that could prevent diarrhea induced by radiotherapy was reported by two studies (Delia et al. 2002; Wang et al. 2019). Similar result was reported in the study by Urbancsek. The study reported that the use of this bacteria helps in reducing the need of anti-diarrheal drug (Urbancsek et al. 2001; Linn et al. 2019). Rauch and their co-workers suggested that risk of gastrointestinal cancer could be decreased by regular intake oral probiotics (Rauch and Lynch 2012; Champer et al. 2018). This similar effect could be achieved by using vaginal probiotics which could reduce the rate of HPV infection and also increase the rate of clearance of the HPV (Champer et al. 2018). The rate of relapse of bacterial vaginosis can also be reduced by using probiotics (Champer et al. 2018). *Lactobacillus iners* is generally associated with high-risk HPV infections. Other lactobacilli, including *L. jensenii*, *L. gasseri*, and *L. crispatus*, present preferably in the healthiest part of the cervix. They can produce antimicrobial substances such as bacteriocin, lactic acid, and hydrogen peroxide. They also compete with the pathogenic bacteria and form barriers to prevent their colonization and adherence on cervix. E6 and E7 are two oncogenes that are encoded by high-risk HPV (Yim and Park 2005). These two genes can suppress p53 and pRB tumor suppressors which is prerequisite for cervical cancer pathogenesis. Lactobacillus supernatants (LS), *L. jensenii,* L. *crispatus,* and *L. gasseri*, treatment leads to downregulation of cyclin A, CDK2, and HPV oncogenes (E6 and E7) which may be beneficial for cervical cancer patients (Wang et al. 2018). Earlier studies reported that *Bifidobacterium adolescentis* exerts an antiviral effect on SiHa cervical cell line (Cha et al. 2012). Treating cells with this bacteria strain are reported to reduce the E6 mRNA and protein levels expression. It was also reported that *L. gasseri* has a smaller inhibitory impact on the E6 gene alone and *L. crispatus* has an inhibitory effect on the expression of E6 and E7 oncogene at the mRNA level (Li et al. 2019). A

study identified the impacts of probiotic strains on the cytological quality of cervical smears and clearance of high-risk human papillomavirus in cervix (Ou et al. 2019). Study also reported the anti-inflammatory role of *Lactobacillus plantarum* NK3 and *Bifidobacterium longum* NK49 which suppress NF-κB that was induced by HPV infection in the mice vagina and uterus (Kim et al. 2019).

17.7 Conclusion

It can be postulated that the use of synbiotic therapy with other conventional treatments of cancer can help in reducing the side effects of those treatments. Synbiotics clearly represent a novel and popular therapeutic approach to cervical cancer prevention because they are cost-effective, with little side effects, easier to administer unlike the current complicated treatment regime for high-grade cervical cancer, which involves a surgical method that carries significant risk to future reproductive side effects. Thus, it appears that modulation of vaginal microbiota with the application of synbiotics can prevent HPV and such application would be a safe and cost-effective way to protect the reproductive health of women.

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Conflicts of Interest Statement There are none.

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