



RESEARCH ARTICLE

PROBIOTICS AS A POTENTIAL ADJUVANT THERAPY FOR THE TREATMENT OF
COLORECTAL CANCER

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ARTICLE INFO

Article History:

Received 07th February, 2016
Received in revised form
21st March, 2016
Accepted 25th April, 2016
Published online 31st May, 2016

Key words:

Colorectal cancer,
Probiotics,
5-Fluorouracil,
Oxaliplatin,
Biomarkers.

ABSTRACT

Colorectal cancer (CRC), also known as colon cancer or bowel cancer is a cancer resulting from uncontrolled cell proliferation or growth in the colon or rectum or in the appendix. 5-fluorouracil (5-FU) and Oxaliplatin are most frequently prescribed drugs for treatment of CRC. But many side effects like mucositis, diarrhea, nausea; vomiting, neurotoxicity, hand foot syndrome, tinnitus, and myelosuppression, anaphylactic reaction etc are the problems with chemotherapy. Probiotics are bacterial cultures comprising of potentially beneficial bacteria or yeast, administered in adequate amounts confer a health benefit on the host. Lactic acid bacteria (LAB) are the most common microbes used. Prebiotics are non-digestible fibre compounds that act as substrate for the probiotics and stimulates the growth of useful bacteria of large intestine and probiotics. The ingestion of probiotics, prebiotics or combinations of both (synbiotics) represents a novel new therapeutic option. Probiotics and prebiotics act to alter the intestinal microflora by increasing concentrations of beneficial bacteria such as *Lactobacillus* and *Bifidobacteria*, and reducing the levels of pathogenic micro-organisms. This strategy has the potential to inhibit the development and progression of neoplasia via mechanisms including: decreased intestinal inflammation, enhanced immune function and anti-tumorigenic activity, binding to potential food carcinogens including toxins found in meat products, and a reduction in bacterial enzymes which hydrolyze precarcinogenic compounds, such as beta-glucuronidase. The present review is an attempt to explore the role of combination of probiotics with the drugs to observe the efficiency profile of the drugs in the management of CRC.

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Citation: Nasreen Sulthana and Vijay K. Chidrawar. 2016. "Probiotics as a potential adjuvant therapy for the treatment of colorectal cancer", *International Journal of Current Research*, 8, (05), 31920-31925.

INTRODUCTION

Cancer can be defined as a disease in which a group of abnormal cells grow uncontrollably by disregarding the normal rules of cell division. Normal cells are constantly subjected to signals that dictate whether the cell should divide, differentiate onto another cell or die. Cancer cells develop a degree of autonomy from these signals, resulting in uncontrolled growth and proliferation. If this proliferation is allowed to continue and spread, it can be fatal. In fact, almost 90% of cancer-related deaths are due to tumor spreading- a process called metastasis. Colorectal cancer (CRC), commonly known as colon cancer or bowel cancer, is a cancer from uncontrolled cell growth in the colon or rectum (parts of the large intestine), or in the appendix.

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CRC results from the cumulative effects of sequential genetic alterations in proto-oncogenes, tumor suppressor genes and DNA repair genes. In sporadic (non hereditary) CRC, these alterations are acquired, and are likely to be caused by exogenous and endogenous carcinogens. In contrast, in cancer syndromes such as familial adenomatous polyposis (FAP) and hereditary non-polyposis CRC (HNPCC), critical genetic alterations that predispose to malignancy are inherited. For example, in FAP, a germ line mutation in the APC gene (Grodin *et al.*, 1991) which occurs in every cell predisposes to adenomatous polyps, while in HNPCC, mutations in DNA repair genes result in a more rapid accumulation of genetic alterations which increases the risk of polyp formation (Oving & Clevers, 2002). There are three main approaches to treat established cancer i.e. surgical excision, irradiation and chemotherapy (Rose and Harris, 2012). The relative value of each of these approaches depends on the type of tumor and the stage of its development.

Drugs Prescribed in Colorectal Cancer

The drugs most often used for bowel cancer treatment are (FDA approved drugs) (Douillard *et al.* 2000):

- Fluorouracil (also called 5FU) often given with a vitamin called folinic acid.
- Oxaliplatin (Eloxatin).
- Capecitabine
- Uftoral (also called tegafur with uracil)
- Irinotecan (Campto)
- Bevacizumab (Avastin),
- Cetuximab (Erbix)

Because the mechanism of chemotherapy is to kill rapidly dividing cancer cells, it also kills other rapidly dividing healthy cells in our bodies, such as

- Membranes lining the mouth,
- Lining of the gastrointestinal tract (mucositis)
- Hair follicles causing alopecia
- Bone marrow (bone marrow suppression) with decreased leucocyte production and thus decreased resistance to infection.
- Gonads causing sterility.

As a result, the side effects of chemotherapy relate to these areas of damaged cells.

Chemotherapy cause severe unwanted effects like vomiting, nausea and diarrhea. In addition, patients may be unusually tired, and there is an increased risk of infection. Neuropathy (tingling or numbness in feet or hands) may also occur with some drugs.

- Mucositis, also referred to as mucosal barrier injury, is one of the most debilitating side effects of radiotherapy and chemotherapy treatment. It is associated with pain, bacteremia, and malnutrition. Furthermore, mucositis is a frequent reason to postpone chemotherapy treatment, ultimately leading towards a higher mortality in cancer patients (Vliet *et al.*, 2010).

Pathophysiology of colon cancer

Colorectal carcinogenesis is a multistep process involving the inactivation and activation of a variety of well-defined tumor suppressor genes, oncogenes and DNA mismatch repair genes. Colon cancer arises from mucosal colonic polyps. Histologically colonic polyps are classified as:

Hyperplastic: Hyperplastic polyps are found in the distal colon and rectum. They have no malignant potential, which means that they are no more likely than normal tissue to eventually become a cancer. They are serrated polyps. They have three histologic patterns of growth: micro vesicular, goblet cell and mucin poor.

Adenomatous: Adenoma is a benign tumor of glandular tissue, such as the mucosa of stomach, small intestine, and colon, in which tumor cells form glands or gland like

structures. In digestive tract or in other hollow organs, the adenoma which is a benign tumor from glandular tissue of epithelium penetrates into the lumen forming adenomatous polyp or polypoid adenoma. The adenoma can be pedunculated with lobular head and a slender stalk or sessile with broad base.

- Colon cancer arises from adenomatous polyps as these polyps are malignant.

Colon carcinogenesis occurs through distinct stages. The first one is the hyper proliferation of epithelium accompanied by deregulation of the expression of several tumor suppressor genes and oncogenes. It results from sequential genetic alterations in proto-oncogenes, tumor suppressor genes and DNA repair genes. In sporadic or non-hereditary CRC these alterations are caused by exogenous and endogenous carcinogens. In contrast, in hereditary cancer syndromes such as familial adenomatous polyposis (FAP) and hereditary non-polyposis CRC (HNPCC), critical genetic alterations that lead to malignancy are inherited. For example, in FAP, a germline mutation in the APC gene which occurs in every cell predisposes to adenomatous polyps, while in HNPCC, mutations in DNA repair genes result in a more rapid accumulation of genetic alterations which increases the risk of polyp formation (Fodde, 2002).

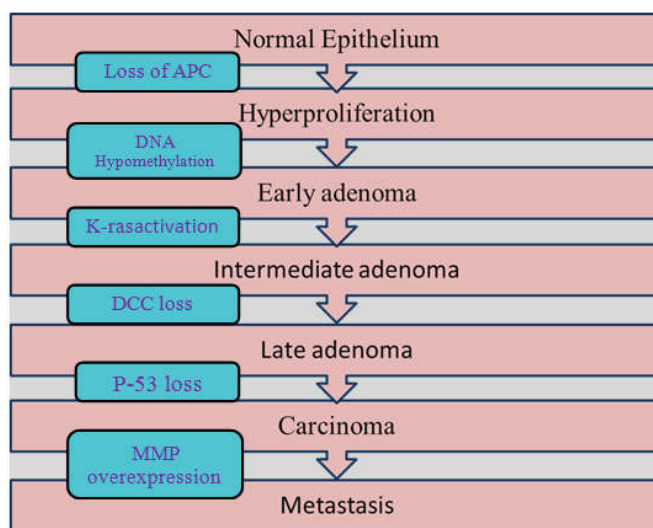


Figure 1. Stages of colon carcinogenesis

Patients with FAP (Familial Adenomatous Polyposis) develop huge number of adenomatous polyps throughout the colon beginning after puberty and develop colon cancer. This syndrome is inherited as a classic mendelian autosomal-dominant single gene. FAP is mainly caused due to the germline mutation of APC (Adenomatous polyposis coli) gene which is located on chromosome 5q. This mutation is responsible for development of number of polyps in the colon forming colonic adenomas following the loss of second APC allele (Cappell, 2005). HNPCC was shown to be caused by mutations of one of the mismatch repair genes, including hMSH2, hMSH6, hMLH1, hMLH3. Germ line mutations of the hMLH1 and hMSH2 genes account for most of the cases. Mismatch repair enzymes, encoded for by mismatch repair

genes, normally recognize errors in nucleotide matching of complementary chromosome strands and initiate segmental excision of the newly synthesized strand to ensure faithful strand replication. Cells with mismatch repair gene mutations cannot repair spontaneous DNA errors and progressively accumulate mutations throughout the genome with succeeding DNA replications. This progressive accumulation leads to genetic mutations in oncogenes and tumor suppressor genes that can result in colon cancer (Cappell, 2005).

Metastasis

Metastasis is the spread of a disease from one organ or part to another non-adjacent organ or part. The anatomical site where the cancer or tumor cell progression takes its origin is called primary tumor. Some cancer or tumor cells break from primary tumor and infiltrate or penetrate to other organ where they are called as secondary tumors.

Routes of metastasis

Metastasis occurs by four routes

- Spread into body cavities: This occurs by the seeding surface of the peritoneal, pleural, pericardial or subarachnoid spaces. For example, carcinoma of the ovary spreads transperitoneally to the surface of the liver.
- Invasion of lymphatics: This is followed by the transport of tumor cells to regional nodes and ultimately to other parts of the body through lymph.
- Hematogenous spread: This occurs through blood. Because of their thinner walls veins are more frequently invaded than arteries and metastasis follows the pattern of the venous flows.
- Transplantation: it involves carrying of tumor cells mechanically through surgical instruments during operation or by needles during diagnostic procedures (Hunters, 2008)

Metastasis of Colorectal Cancer

Colorectal cancer metastasizes preferentially to liver, lung and bone marrow.

Liver Metastasis: SDF-1, CXCR4 are over expressed in colorectal cancer. CXCR4 stimulates migration and invasion of cancer cells (Murakami *et al.*, 2013). Higher expression of SDF-1 in the liver may selectively attract the cells with increased levels of CXCR4. Therefore, the foremost organ affected by metastasis of colorectal cancer is liver. Alternate mechanism: Predominant sites of metastasis merely reflect the first pass of cancer cells in the circulation and entrapment in local capillaries.

Lungs Metastasis: Lung metastasis of lung is due to activation of CXCR3 in colorectal cancer cells (Walser *et al.*, 2006). The ligands for CXCR3 are found in increased levels in lungs thereby causing lung metastasis (Murakami *et al.*, 2013).

Bone Marrow Metastasis: Bone marrow metastasis is may be due to CXCR4 (Murakami *et al.*, 2013).

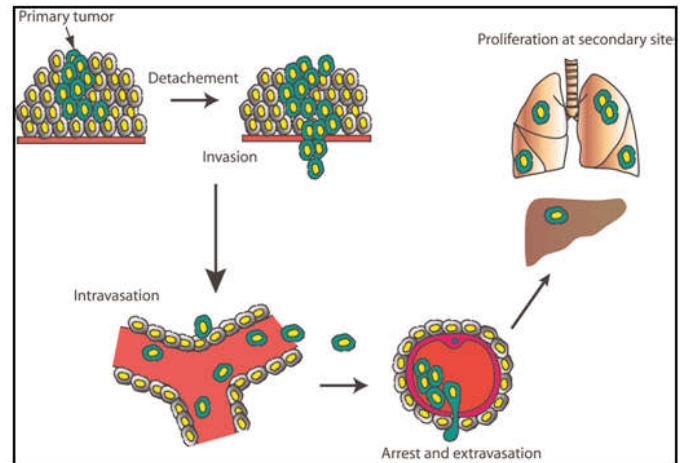


Figure 2. Metastatic process

Role of Biomarkers in Colorectal Cancer

Biomarkers: Biomarkers are the identifiable characteristics which are objectively measured and evaluated as an indicator of normal biological processes. They are used to evaluate normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention (Bhatt *et al.* 2011). Genetic alterations in cells lead to disturbances in molecular pathways regulating cell growth, survival, and metastasis. When such changes occur in majority of patients with a specific type of tumor, these can be used as biomarkers for detection, developing targeted therapies and also in predicting responses to various treatments. Biomarkers are invaluable tools for cancer detection, diagnosis, patient prognosis and treatment selection. These can also be used to localize the tumor and determine its stage, subtype, and response to therapy. Identification of such signature in surrounding cells or at more distal and easily sampled sites of the body like cells in the mouth (instead of lung) or urine (instead of urinary tract) can also influence the management of cancer (Malati, 2007)

The biomarkers which are considered in this review are:

- Alkaline phosphatase (ALP)
- Adenosine deaminase (ADA)
- C - reactive protein. (CRP)
- CA.19.9

Probiotics

Probiotics are defined as “live microorganisms that when administered in adequate amounts confer a health benefit on the host” (Boyle, 2006). Probiotics are bacterial cultures comprising of potentially beneficial bacteria or yeast. Lactic acid bacteria (LAB) are the most common microbes used. Cancer-causing chemicals (carcinogens) can be ingested or generated by metabolic activity of microbes that live in the gastrointestinal tract (Parvez *et al.*, 2006).

Benefits

There are a wide range of potentially beneficial medicinal uses for probiotics.

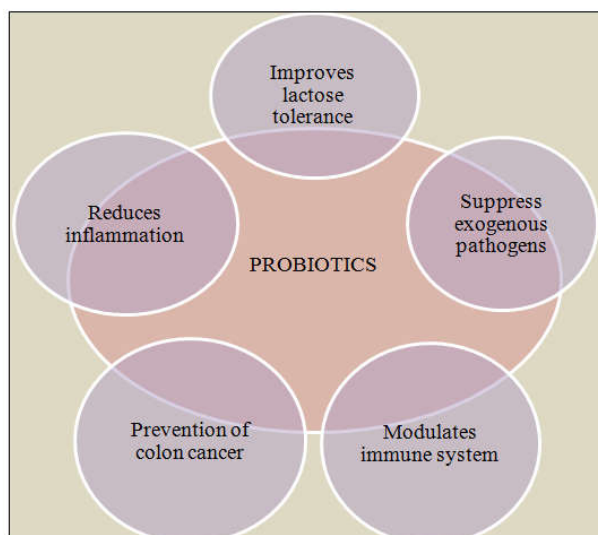


Fig. 3. Various health benefits from probiotics consumption

Role of probiotics in reducing biomarker levels

At present, because of dissatisfaction with high costs and potentially hazardous side-effects of chemotherapeutic agents there is an immediate need to improve drug potency and suppress the toxicity profile of the available drug sources which already have proven their effectiveness for treatment of CRC. CRC has several etiological factors of which activities of certain colonic microbiota are considered as one. Therefore, manipulation of their activities in colon by using probiotics may be useful to CRC risk (Verbeke *et al.*, 2008). Probiotics are defined as living micro-organisms which upon ingestion in certain numbers provides health benefits beyond inherent general nutrition (Guarner and Schaafsma, 1998; Ouwehand *et al.*, 2002). The vast majority of studies on the anticancer effects deal with colorectal cancer (CRC), although some have investigated breast (Veer *et al.*, 1989) and bladder cancer (Ohashi *et al.*, 2002). All biomarker level including ALP, ADA, CRP and CA.19.9 considered in this review are significantly increased in patients with CRC.

Effect on ALP

Probiotics shows protective effects by various mechanisms. Serum ALP levels are frequently elevated in patients with metastatic colorectal cancer (CRC) due to the increased de novo synthesis of the enzyme and subsequent regurgitation into the serum (Saif *et al.*, 2005). The fall in the ALP level by these various drug combinations is because of their well-established mechanisms to CRC.

Effect on ADA

Human adenosine deaminase-ADA (E.C. 3.5.4.4.) exists in various molecular forms, with different molecular weights. There are 2 isoforms of ADA: ADA1 and ADA2. The plasma ADA2 isoform increases in most cancers. ADA2 is not ubiquitous but co-exists with ADA1 only in monocytes-macrophages. It plays an important role as a key enzyme involved in the salvage of purine nucleosides and recycling of

purines. Numerous studies have revealed the highest activity and substantial molecular heterogeneity of intestinal adenosine deaminase. Beside of soluble ADA form, a particulate-membrane bound form was isolated also from normal intestine, but a cancer-specific form was isolated in some colorectal tumors. The enzyme is particularly sensitive to stimulation by the growth factors and cytokines during rapid tissue proliferation. The treatment of colon carcinoma cells with deoxycoformicine, an ADA inhibitor, resulted in inhibition of cell growth. The highest ADA activity obtained from mucosa adjacent to carcinoma may suggest its role in colon carcinoma progression and invasion. Probiotics decreases the level of ADA because of their anti-inflammatory action i.e. they suppress inflammatory mediators (Lee *et al.*, 2010).

Effect on CRP

Chronic inflammation is one of the major causes of CRC (Gunter *et al.*, 2006). Physiologically, CRP binds to phosphocholine expressed on the surface of dead or dying cells and activates the complement system (Vrese *et al.*, 2005). It binds to phosphocholine on microbes and damaged cells and enhances phagocytosis by macrophages. Therefore, it plays a role in clearing the necrotic and apoptotic cells. The synthesis of CRP is up regulated by proinflammatory cytokines as interleukin-1, interleukin-6, and tumour necrosis factor, which act as autocrine growth factors for neoplasm (Mazhar & Ngan, 2006). The protective effect of probiotics in this regards is because of LAB. Direct interaction between *Lactacid Bacillus* (LAB) and Natural killer (NK) cells may occur in the epithelium, where NK cells reside among the intraepithelial lymphocytes possibly by interaction between bacterial CpG DNA and TLR9 (Toll like receptor 9), which is present in NK cells (Watzl, 2008) resulting in anti-inflammatory action.

Effect on CA 19-9

CA 19-9 also referred to as sialyl Lewis-a (sLea), is expressed on the surface of cancer cells as a glycolipid and O-linked glycoprotein. Patients with other gastrointestinal tumors like esophageal, gastric, biliary and pancreatic cancer also express CA 19.9 in their tissues and serum. CA 19-9 is derived from an aberrant pathway during production of its normal counterpart disialyl Lewis-a which has one extra sialic acid residue attached through a 2→6 linkage. Disialyl Lewis-a, ligand for monocytes and macrophages is normally expressed on the epithelial surface of digestive thus helping in immune surveillance. Epigenetic silencing of the gene for 2→6 sialyl transferase during early stages of carcinogenesis leads to abnormal synthesis and accumulation of CA 19-9. sLea may also play a role in cancer invasion/metastasis as it is known to be a ligand for endothelial cell E-selectin responsible for cell adhesion (Ballehaninna & Chamberlain, 2011). Anaerobic breakdown of prebiotics (FOS) and their subsequent fermentation by probiotics not only enhances the growth of LAB but also leads to the production of SCFA (Short chain fatty acids) like butyrate, acetate and propionate. SCFAs are the end products of the physiological bacterial fermentation of alimentary fibres in the colonic lumen (Ballehaninna & Chamberlain, 2012). SCFA play a critical role in maintaining homeostatic cell turnover in the colonic epithelium. Of these,

butyrate, an inhibitor of histone deacetylase has the ability to inhibit cell proliferation and induces apoptosis of transformed cells (Zhang *et al.*, 2010). Several gene products are also known to be important in controlling the apoptotic process. The imbalance of expression of anti and pro-apoptotic proteins after the stimulus is one of the major factors that contributes to the disturbance in the apoptotic process leading to cancer. Butyrate induces both mitochondria-mediated apoptosis and caspase-independent apoptosis (Shao *et al.*, 2004). Mitochondria are involved in a variety of key events leading to apoptosis, such as releasing of caspase activators, changes in electron transport, the production of ROS, and participation in regulation of both pro- and anti-apoptotic Bcl-2 family proteins. It has been recognized that the Bcl-2 family plays crucial roles in regulating apoptosis by functioning as promoters (e.g., Bax) or inhibitors (e.g., Bcl-2) of cell death. The Bcl-2 family proteins, whose members may be antiapoptotic or pro-apoptotic, regulate cell death by controlling the mitochondrial membrane permeability during apoptosis. Bcl-2 is a potent anti-apoptotic factor, whereas Bax is pro apoptotic factor. Bax is localized mostly in the cytoplasm, but redistributes to mitochondria in response to stress stimuli. A decrease in the levels of Bcl-2 and an increase in Bax lead to the loss of mitochondrial transmembrane potential, a key event in the induction of apoptosis. Treatment of butyrate leads to a shift from an anti-apoptotic to a pro-apoptotic state, which results in the activation of caspase-3 that causes fall in the CRC cell count and subsequent less serum level of CA 19-9.

High red meat consumption remains one of the major reasons for the development of CRC. This is due to the presence of heme iron and fat in red meat. Iron and fat, both decrease manganese superoxide dismutase (SOD) activity in the cells lining the colon. Manganese SOD is an antioxidant enzyme that protects mitochondria from oxygen radical damage. Manganese SOD has tumor suppressive activity and has been found lacking in most colon tumors. Some meats are cooked at high temperatures, resulting in the production of heterocyclic amines and polycyclic aromatic hydrocarbons both of which are believed to have carcinogenic properties. In addition, certain mutagenic xenobiotics, after absorption, are detoxified in the liver by conjugation with glucuronic acid and are again released into intestine as glucuronide conjugates. In GIT, bacteria like enterobacteria and clostridia cause regeneration of these toxic mutagenic aglycones again from the conjugates by liberating enzymes like β -glucuronidase, nitroreductase and azoreductase which are liable to cause cancer (Fotiadis, 2008). The probiotics are capable of altering certain enzymes (such as β -glucuronidase and nitro-reductase) that turn procarcinogens into carcinogenic agents by neutralizing the bad bacteria enzymes. LAB and certain other strains like *Bifidobacteria* have ability to inactivate the polycyclic aromatic hydrocarbons and heterocyclic amines by conjugation with glutathione with the help of the enzyme glutathione S-transferase (GST) found in the liver and other tissues including gut. Probiotic ingestion induces the chemo preventive enzyme GST in the colon. Probiotics are also able to modulate several intestinal functions such as detoxification, colonic fermentation, transit and immune status which may contribute to the development of colon cancer (Sanders 2003).

Conclusion

Studies on probiotics in colorectal cancer are promising. Due to modern diets and lifestyle, as well as environmental factors such as pollution and the irresponsible overuse of antibiotics, the beneficial bacteria in our micro biome is at risk which can lead to an increased incidence in metabolic and inflammatory chronic diseases. Even simple aging gradually shifts our intestinal bacterial population towards a disease-promoting, rather than a disease-preventing state. Research is catching up with this traditional wisdom in the form of accelerated scientific investigations into the broad spectrum health benefits of probiotics. This new science, known as pharmabiotics, uses probiotic organisms as natural pharmaceutical agents in the treatment and prevention of disease along with promoting longevity. In conclusion, our review supports the use of probiotics as an adjuvant therapy to chemotherapy thereby adding benefit to the present chemotherapy and hence improving the therapeutic strategies and quality of life in colorectal cancer patients.

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