Probiotics as Potential Therapeutics for Colorectal Cancer

Myung Jun Chung*, Byung Chull An, Yongku Ryu, Sunwoong Hong and Daebeom Kwon

R&D Center, Cell Biotech, Co., Ltd., Korea

*Corresponding author: Myung Jun Chung, R&D Center, Cell Biotech, Co., Ltd., 50, Aegibong-ro 409 beon-gil, Gaegok-ri, Wolgot-myeon, Gimpo-si, Gyeonggi-do, Korea.

To Cite This Article: Myung Jun Chung*, Byung Chull An, Yongku Ryu, Sunwoong Hong, Daebeom Kwon. Probiotics as Potential Therapeutics for Colorectal Cancer. 2020 - 9(2). AJBSR.MS.ID.001362. DOI: 10.34297/AJBSR.2020.09.001362.

Received: May 27, 2020; Published: June 10, 2020

Abstract

Much health benefits by lactic acid bacteria (LAB) have been observed via in-vivo trials. LAB’s health benefits are described such as improving of intestinal microbial balance between probiotics and harmful bacteria, protecting of pathogen infection and modulating of host’s immunity in gut. Colorectal cancer (CRC) located in intestine and its development leads to invade or spread to other parts of the body result in death. Recent many publications about LAB have demonstrated the immense potential as alternative bio-therapeutics. Actually, LAB have been reported to be the safe bio-therapeutic with positive effects against various cancer types including CRC. In this review, we discuss the evidences of beneficial effects of LAB application and its molecular mechanisms.

Keywords: Lactic acid bacteria; Probiotics; Colorectal cancer; Bio-therapeutics; Anti-cancer activity

Introduction

Colorectal cancer (CRC) develops in the intestine, from where it can invade or spread to other parts of the body and cause death if untreated [1]. Treatments include combinations of surgery, radiation therapy, chemotherapy, and targeted therapy [2,3]. Chemotherapy involves injection of natural, synthetic, or biological substances to suppress or prevent progression of CRC; however, many chemotherapy agents are themselves highly cytotoxic, even novel agents that have reached the clinical or commercial stages of development [4]. Since the first biopharmaceutical approval of recombinant insulin by the federal drug association (FDA) in 1982, biopharmaceuticals have been used successfully as therapeutic agents because they have fewer non-clinical and clinical toxicity failures than chemical therapeutics [5,6].

To overcome the limitations and safety concerns surrounding therapeutic agents, recent reports suggest a novel approach that involves screening lactic acid bacteria (LAB) for use as biopharmaceutical factories; this is because these human intestinal microbes, which are generally regarded as safe, can be engineered to secrete proteins with anti-CRC effects [7,8]. For over at least 4,000 years, LAB have been used to ferment foods such as cheese, yoghurt, and kimchi [9,10]. In general, these microbes are Gram-positive, non-spore-forming, non-respiring cocci or rods that produce lactic acid as the major end product during fermentation of carbohydrates. LAB are an effective chemo preventive food ingredient with positive effects against many cancer types [11,12]; as such, they have attracted much interest due to their ability to reduce cancer risk [13,14]. A recent report demonstrates that LAB exert anti-CRC properties by suppressing tumor initiation or progression via various pathways [15], although most studies have only investigated the relationship between LAB and cancer. In addition, studies show that cell wall derivatives of LAB suppress tumorigenesis [16-18].

In this review, we discuss recent insights into the cellular and molecular mechanisms underlying the anti-cancer effects of LAB, including cell cycle arrest, apoptosis, immune responses, inflammatory responses, antioxidant DNA damage, and epigenetics.

Cell Cycle Arrest

Lactobacillus reuteri (L. reuteri) may suppress CRC proliferation by reducing expression of Cox-2 and cyclin D1 [19]. Lactobacillus rhamnosus (L. rhamnosus)-derived p8 protein exhibits anti-
proliferative activity against CRC cell line DLD-1. P8 induces p21 very efficiently, resulting in reduced expression of cyclin B1/CDK1 protein, thereby arresting the cell cycle at G0/M phase. The next step for the anti-cancer gene therapy using P8 gene will be to design effective vectors that enable its delivery into tumor cells [20]. In addition, *Lactobacillus paracasei* (*L. paracasei*) effectively arrests the cell cycle at G1 phase by inhibiting cyclin E1 and enhancing p27; these effects are mediated by the mTOR/4EBP1 signaling pathway [21]. *Lactobacillus plantarum* (*L. plantarum*) triggers cell cycle arrest in late G1 phase by activating p53 to mediate transcriptional upregulation of p21 [22]. *Enterococcus faecalis* (*E. faecalis*) and *Staphylococcus hominis* (*S. hominis*) cause a significant increase in arrest of human breast cancer cells (MCF-7) at G1/G0 phase and induce cytotxic effects [23]. Finally, *Lactobacillus* strain-derived exopolysaccharides induce G1/G0 phase arrest and apoptosis of HT-29 cells [24].

**Apoptosis**

Apoptosis is a process of genetically programmed cell death that plays a key role in regulating cell proliferation [25,26]. Apoptosis occurs not only during development and senescence, in which it plays a role in regulating cell populations in tissues, but also during defense response such as immune reactions to damage caused by disease or cytotoxic agents [27]. Recent reports demonstrate that LAB play a role in regulating cell apoptosis through various pathways, thereby acting as critical components that prevent CRC. For example, *Lactobacillus acidophilus* (*L. acidophilus*) effectively increases apoptosis and reduces carcinogenesis in mice [28]. *L. reuteri* significantly down-regulates expression of nuclear factor-kappaB (NF-κB)-dependent gene products that in turn regulate expression of survival genes such as Bcl-2 and Bcl-xL [29]. Other studies report that *L. acidophilus* and *Lactobacillus casei* (*L. casei*) act synergistically to enhance 5-fluorouracil (5-Fu)-mediated apoptosis of CRC cell line LS513 [30].

**Immune Responses**

Alterations in the LAB-derived microbiota in the gastrointestinal tract have a marked effect on host immune responses. M cells in the human gut are crucial because they have the capacity to transport macromolecules, antigens, microorganisms, and inert particles from the gut lumen into lymphoid tissue via absorptive endocytosis. When antigenic molecules cross the intestinal barrier, they stimulate the host innate and adaptive immune systems [31]. Immunity is highly specific and destroys individual invading pathogens. In addition, long-lasting pathogen-specific protective memory enables the adaptive immune system to attack and destroy pathogens when re-encountered [32]. Lymphocytes, particularly B cells and T cells, mediate adaptive immune responses by recognizing antigens via specific receptors. Recent studies implicate LAB in immune responses critical for CRC prevention and therapy [33]. LAB-mediated effects on the gut microbiome seem to focus specifically on responses to immune checkpoint blockade [34-37].

**Inflammatory responses**

Inflammatory molecules are involved in various steps of carcinogenesis; thus, those with inflammatory conditions such as ulcerative colitis are at high risk of developing CRC [37]. *L. plantarum* reduces expression of proinflammatory cytokines and inflammatory genes and suppresses inflammatory markers in a colitis mouse model [38]. In addition, *L. rhamnosus* suppresses tumor development, progression, and volume by inactivating NF-κB; this in turn dampens proinflammatory responses and angiogenesis, both of which play central roles in tumor development and progression [39]. Suppression of Treg in splenocytes and splenocytes by L. casei BL23 may be associated with the Th17 T-cell biased immune response which accompanied the expression of regulatory cytokines in mice (IL-6, IL-17, IL-10, and TGF-β) [40].

**Antioxidant DNA damage**

Reactive oxygen species (ROS) act as both important signaling molecules and as mediators of inflammatory responses. Although ambient levels of ROS are important for cellular homeostasis, excess ROS overwhelm the antioxidant machinery and cause inflammatory tissue injury [41]. LAB exert metabolic antioxidant activity by scavenging ROS, inhibiting related enzymes, and reducing/inhibiting the activity of ascorbate autoxidation in the intestine [42]. Studies suggest that ROS play a key role in IBD and CRC [43,44]. *In vitro* studies of the colon cell line (HT-29) suggest that the antioxidant activity by LAB (mediated by reducing/inhibiting ROS) plays a key role in CRC [45,46]. For example, *Bifidobacterium longum* (*B. longum*) and *L. acidophilus* significantly reduce peroxidation of linoleic acid [47], and *L. plantarum* modulates development of 1,2-dimethyl benzanthracene (DMH)-induced colon carcinogenesis in rats by altering lipid peroxidation and antioxidant enzyme activity [48]. *Streptococcus thermophilus* (*S. thermophilus*) prevents oxidative damage by releasing ROS-protective factors. Furthermore, whereas the obligatory homofermentative lactobacilli display high antioxidant activity, this property is highly strain-dependent among facultative and obligate heterofermentative lactobacilli [49,50]. Taken together, these studies suggest that LAB are key mediators of antioxidant activity, which may help to prevent CRC.

**Epigenetics**

Epigenetic events such as DNA methylation, histone tail modifications, chromatin remodeling, and non-coding RNA molecules can alter expression of specific genes in cancer cells without necessarily altering their DNA sequences [51]. Recent reports have examined the role of LAB-derived epigenetic alterations in cancer cells [52,53]. For example, probiotic-mediated butyrate specifically induces expression of histone deacetylase
inhibitors (HDACi), well-known targets of epigenetic drugs for cancer therapy. Metabolites of LAB, such as short-chain fatty acids (SCFA), biotin, folic acid, and other bioactive molecules, have diverse effects, including altering the composition of the microbiota, regulating epithelial cell barrier function, modulating immune responses, and epigenetic control of host cell responses in the intestine [54]. Indeed, SCFAs show anti-inflammatory properties and can increase the numbers of colonic regulatory Tregs, thereby providing protection against colitis [55].

Conclusions

LAB are safe active ingredients found in functional foods. Therefore, people have traditionally believed that such foods have nutritional benefits. Additionally, numerous in vitro and in vivo studies provide evidence of the beneficial effects of LAB against various cancer types. The effects are mediated via diverse mechanisms, including altering the composition of the gastrointestinal microflora, enhancing host immune responses, and exerting anti-inflammatory and anti-proliferative activity. In this review, we described the results of studies related to the anti-cancer effects of LAB. The secretome of the intestinal microbiota allows humans to utilize various dietary ingredients during digestion. We described studies that have examined the direct mechanisms and molecular targets of LAB-derived substances; however, more studies are needed to gain a deeper understanding of the underlying mechanisms based on the phenotype of the anti-cancer effects of LAB.

In conclusion, current cancer therapies have limited efficacy because they are highly toxic to both cancer cells and normal tissues. However, numerous reports show that LAB have chemopreventive effects, even though the magnitude of the effects (therapeutic activity) does not match that of chemical drugs. However, not only can LAB be used as a natural adjuvant for chemotherapy, but they can be engineered to deliver therapeutic payloads. Many creative approaches have been adopted to exploit natural bacterial processes or to harness bacteria as therapy vectors and cancer cell destroyers. Safety problems associated with genetic engineering need to be overcome; however, we believe that LAB can be an important new tool to add to the cancer therapy toolbox.

Acknowledgments

This study was supported by the World Class 300 Project, funded by the Small and Medium Business Administration (SMBA, S2367890 [S2416714]), Korea.

Author contributions

Byung Chull An: Organizing and manuscript writing
Yongku Ryu: Cell cycle arrest and apoptosis
Sunwoong Hong: Immune responses and inflammatory response

Daeboom Kwon: Antioxidant DNA damages and epigenetics

Myung Jun Chung: Supervisor of this study

References

1. (2007) Defining Cancer. National Cancer Institute.
2. (2014) Colon Cancer Treatment (PDQ®).
3. Zhang L, Ren X, Alt E, Bai X, Huang S, et al. (2010) Chemoprevention of colorectal cancer by targeting APC-deficient cells for apoptosis. Nature 464: 1058-1061.
4. Gosses ME, DiMaio JA, Nelson TF (1996) Recombinant protein and therapeutic monoclonal antibody drug development in the United States from 1980 to 1994. Clin Pharmacol Ther 66(6): 608-618.
5. Lowe JA, Jones P (2007) Biopharmaceuticals and the future of the pharmaceutical industry. Curr Opin Drug Discov Develop 10(5): 513-514.
6. Giezen TJ, Mantel Teunwisse AK, Strauss SIJM, Schellekens H, Leufkens HGM, et al. (2008) Safety-related regulatory actions for biologics approved in the United States and the European Union. J Am Med Assoc 300(16): 1887-1896.
7. Lee NK, Paik HD (2017) Bioconversion Using Lactic Acid Bacteria: Ginsenosides, GABA, and Phenolic Compounds. J Microbiol Biotechnol 27(5): 869-877.
8. Gilliland SE (1990) Health and nutritional benefits from lactic acid bacteria. FEMS Microbiol Rev 7(1-2): 175-188.
9. Lee JM, Choi JY, Lee JH, Chang HC, Chung DK, et al. (1999) Cloning and expression of the UDP-galactose-4-epimerase gene (gâE) constituting the gal/lac operon of Lactococcus lactis ssp. lactis ATCC7962. J Microbiol Biotechnol 9: 393-397.
10. Kim JE, Kim JY, Lee KW, Lee HJ (2007) Cancer chemopreventive effects of lactic acid bacteria. J Microbiol Biotechnol 17(8): 1227-1235.
11. Geier MS, Butler RN, Howarth GS (2006) Probiotics, prebiotics and symbiotics: A role in chemo prevention for colorectal cancer?. Cancer Biol Ther 5(10): 1265-1269.
12. Kumar M, Kumar A, Nagpal R, Mohania D, Behare P, et al. (2010) Cancer preventing attributes of probiotics: An update. Int J Food Sci Nutr 61(5): 473-496.
13. Reid G, Jass J, Schubsky MT, McCormick JK (2003) Potential uses of probiotics in clinical practice. Clin Microbiol Rev 16(4): 658-672.
14. Zhong L, Zhang X, Covasa M (2014) Emerging roles of lactic acid bacteria in protection against colorectal cancer. World journal of gastroenterology 20(24): 7878-7886.
15. Fotiadis CI, Stodíns CN, Spyropoulos BG, Zografius ED (2008) Role of probiotics, prebiotics and symbiotics in chemoprevention for colorectal cancer. World J Gastroenterol 14(14): 4653-4658.
16. Park KY, Jeong JK, Lee YE, Daily JW (2014) Health benefits of kimchi (Korean fermented vegetables) as a probiotic food. J Med Food 17(1): 6-20.
17. Chang JH, Shim YY, Cha SK, Chee KM (2010) Probiotic characteristics of lactic acid bacteria isolated from kimchi. J Appl Microbiol 109(1): 220-230.
18. Iyer C, Kosters A, Sethi G, Kunnumakkara AB, Aggarwal BB, et al. (2008) Probiotic Lactobacillus reuteri promotes TNF-induced apoptosis in human myeloid leukemia-derived cells by modulation of NF-kappaB and MAPK signalling. Cell Microbiol 10(7): 1442-1452.
19. An BC, Hong S, Park HJ, Kim BK, Ahn JY, et al. (2019) Anti-colorectal cancer effects of probiotic-derived p8 protein. Genes 10(8): 624.
20. Huang L, Shan YJ, He CX, Ren MH, Tian PL, et al. (2016) Effects of L. paracasei subsp. paracasei X12 on cell cycle of colon cancer HT-29 cells and regulation of mTOR signalling pathway. J Funct Foods 21: 431-439.
21. Lee HA, Kim H, Lee KW, Park KY (2015) Dead Nano-Sized Lactobacillus plantarum InhibitsAzoxymethane/Dextran Sulfate Sodium-Induced Colorectal Cancer in Balb/c Mice. J Med Food 18(12): 1400-1405.

22. Hassanz, Mustafa S, Rahim RA, Isa NM (2016) Anti-breast cancer effects of liva, heat-killed and cytoplasmic fractions of Enterococcus faecalis and Staphylococcus hominis isolated from human breast milk. In Vitro Cell Dev Biol Anim 52(3): 337-348.

23. Di W, Zhang L, Yi H, Han X, Zhang Y, et al. (2018) Exopolysaccharides produced by Lactobacillus strains suppress HT-29 cell growth via induction of G0/G1 cell cycle arrest and apoptosis. Oncol Lett 16 (3): 3577-3586.

24. De Vries EG, Gietema JA, De Jong S (2006) Tumor necrosis factor related apoptosis-inducing ligand pathway and its therapeutic implications. Clin Cancer Res 12(8): 2390-2393.

25. Bucur O, Ray S, Bucur MC, Almasan A (2006)APO2 ligand/tumor necrosis factor-related apoptosis-inducing ligand in prostate cancer therapy. Front Biosci 11: 1549-1568.

26. Norbury CJ, Hickson ID (2001) Cellular responses to DNA damage. Annu Rev Pharmacol Toxicol 41: 367-401.

27. Chen CC, Lin WC, Kong MS, Shi HN, Walker WA, et al. (2012) Oral inoculation of probiotics Lactobacillus acidophilus NCFM suppresses tumour growth both in segmental orthotopic colon cancer and extra-intestinal tissue. Br J Nutr 107(11): 1623-1634.

28. Iyer C, Kosters A, Sethi G, Kummamakarra AB, Aggarwal BB, et al. (2008) Probiotic Lactobacillus reuteri promotes TNF-induced apoptosis in human myeloid leukemia-derived cells by modulation of NF-κB and MAPK signalling. Cell Microbiol 10(7): 1442-1452.

29. Baldwin C, Millette M, Oth D, Ruiz MT, Luquet PM, et al. (2010) Probiotic Lactobacillus acidophilus and L. casei mix sensitize colorectal tumoral cells to 5-fluorouracil-induced apoptosis. Nutr Cancer 62(3): 371-378.

30. V Snoeck, B Goddeeris, Cox E (2005) The role of enterocytes in the intestinal barrier function and antigen uptake. Microbes and Infection 7(7-8): 997-1004.

31. Tan C, Wei H, Sun H, Ao J, Long G, et al. (2015) Effects of dietary supplementation of oregano essential oil to sows on oxidative stress status, lactation feed intake of sows, and piglet performance. BioMed Research International.

32. Gabrilovich D, Pisarev V (2003) Tumor escape from immune response: mechanisms and targets of activity. Curr Drug Targets 4(7): 525-536.

33. Chaput N, Lepage P, Coutzac C, Soularue E, Le Roux, et al. (2017) Baseline mechanisms and targets of activity. Curr Drug Targets 4(7): 525-536.

34. Frankel AE, Coughlin LA, Kim J, Froehlich TW, Xie Y et al. (2017) Metagenomic shotgun sequencing and unbiased metabolomic profiling identify specific human gut microbiota and metabolites associated with immune checkpoint therapy efficacy in melanoma patients. Neoplasia 19(10): 848-855.

35. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, et al. (2018) Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science 359(6371): 97-103.

36. Gupta SC, Kim JH, Prasad S, Aggarwal BB (2012) Chronic inflammation and cancer: A matter of lifestyle. In: Roy S, et al. [Eds.] Chronic Inflammation: Molecular Pathophysiology, Nutritional and Therapeutic Interventions, CRC Press, Boca Raton pp. 153-171.

37. Lee HA, Bong YJ, Kim H, Jeong JK, Kim HY, et al. (2015) Effect of dietary Lactobacillus plantarum in kimchi on dextran sulfate sodium-induced colitis in mice. J Med Food 18: 1073-1080.

38. Brenner DR, Scherer D, Mui R, Schildkraut J, Boffetta P, et al. (2014) A review of the application of inflammatory biomarkers in epidemiologic cancer research. Cancer Epidemiol Biomarkers Prev 23(9): 1729-1751.

39. Lenoir M, Del Carmen S, Cortes Perez NG, Lozano Ojavo D, Muñoz Provencio D, et al. (2016) Lactobacillus casei BL23 regulates Treg and Th17 T-cell populations and reduces DMH-associated colorectal cancer. J Gastroenterol 51(9): 862-873.

40. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB (2014) Reactive oxygen species in inflammation and tissue injury. Antioxid Redox Signal 20(7): 1126-1167.

41. Amaretti A, di Nunzio M, Pompei A, Raimondi S, Rossi M, et al. (2013) Antioxidant properties of potentially probiotic bacteria: in vitro and in vivo activities. Appl Microbiol Biotechnol 97: 809-817.

42. Bruno Bârnea JM, Andrus JM, Libby SL, Klaenhammer TR, Hassan HM (2004) Expression of a heterologous manganese superoxide dismutase gene in intestinal lactobacilli provides protection against hydrogen peroxide toxicity. Appl Environ Microbiol 70(8): 4702-4710.

43. Kulilasar T, Songiçep E, Mikesaar M, Zilmer K, Vihalem M, et al. (2003) Antioxidative probiotic fermentedgoats’ milkdecreases oxidative stress-mediated atherogenicity in human subjects. Br J Nutr 90(2): 449-456.

44. Köller VJ, Marian B, Stikl R, Nersesyan A, Winter H, et al. (2008) Impact of lactic acid bacteria on oxidative DNA damage in human derived colon cells. Food Chem Toxicol 46(4): 1221-1229.

45. Zhong L, Zhang X, Covasa M (2014) Emerging roles of lactic acid bacteria in protection against colorectal cancer. World J Gastroenterol 20(24): 7878-7886.

46. Lin MY, Chang FJ (2000) Antioxidative effect of intestinal bacteria. Bifidobacterium longum ATCC 15708 and Lactobacillus acidophilus ATCC 4356. Dig Dis Sci 45(8): 1617-1622.

47. Kumar RS, Kammani P, Yuvaraj N, Paari KA, Pattukumvar, V, et al. (2012) Lactobacillus plantarum AS1 isolated from south Indian fermented food Kalappam suppresses 1,2-dimethyl hydrazine (DMH)-induced colorectal cancer in male Wistar rats. Appl Biochem Biotechnol 166(3): 620-631.

48. Annuk H, Shchepevtova J, Kulilasar T, Songiçep E, Zilmer M, et al. (2003) Characterization of intestinal lactobacilli as putative probiotic candidates. J Appl Microbiol 94: 403-412.

49. Kumar M, Kumar A, Nagpal R, Mohania D, Behare P, et al. (2010) Cancer-preventing attributes of probiotics: an update. Int J Food Nutr Sci 61(5): 473-496.

50. Migliore M, Migheli F, Spisni R, Coppèd F (2011) Genetics, cytogenetics, and epigenetics of colorectal cancer. J Biomed Biotechnol 2011: 792362.

51. Berni Canani R, Di Costanzo M, Leone L (2012) The epigenetic effects of butyrate: potential therapeutic implications for clinical practice. Clin Epigenetics 4: 4.

52. Acharya MR, Spärreboom A, Venitz J, Figg WD (2005) Rational development of histone deacetylase inhibitors as anticancer agents: a review. Mol Pharmacol 68(4): 917-932.

53. Liciardi PV, Wong SS, Tang ML, Karagiannis TC (2010) Epigenome targeting by probiotic metabolites. Gut Pathog 2(1): 24.

54. Nagpal R, Wang S, Ahmadi S, Hayes J, Gagliano J, et al. (2018) Human-origin probiotic cocktail increases short-chain fatty acid production via modulation of mice and human gut microbiome. Sci Rep 8(1): 12649.

55. Bultman SJ (2017) Interplay between diet, gut microbiota, epigenetic events, and colorectal cancer. Mol Nutr Food Res 61(1).