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Review Article

Probiotics as Potential Therapeutics for Colorectal Cancer

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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 G_2/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 G_1 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 G_1 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 G_0/G_1 phase and induce cytotoxic effects [23]. Finally, *Lactobacillus* strain-derived exopolysaccharides induce G_0/G_1 cycle 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 adsorptive 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 suppress inflammatory markers in a colitis mouse model [38]. In addition, *L. rhamnosus* suppresses tumor development, progression, and volume by inactivating NF-kB; 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 (HT29) 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-oxidative 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 chemo preventive 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.

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Author contributions

Byung Chull An: Organizing and manuscript writing

Yongku Ryu: Cell cycle arrest and apoptosis

Sunwoong Hong: Immune responses and inflammatory response

Daebeom Kwon: Antioxidant DNA damages and epigenetics

Myung Jun Chung: Supervisor of this study

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