

Genetic engineering of a probiotic-based drug delivery system for colorectal cancer therapy

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Abstract

Food-grade bacteria, including lactic acid bacteria (LAB), are safe to ingest and are not associated with development of disease. The impact of LAB on health has been studied in depth from a clinical perspective. Evidence suggests that LAB benefit health in several ways, including improving the balance between probiotic and harmful bacteria in the intestine, protecting against pathogen infections, and modulating host immunity in the gut. Recent publications show that LAB are safe bio-therapeutics that exert positive effects against various cancer types, including colorectal cancer (CRC). CRC develops in intestine and, unless treated early, will invade the surrounding tissue and spread to other parts of the body. One intrinsic advantage of food-grade LAB is that they can be applied easily as oral medications and act as vehicles for bio-therapeutics, which can be delivered directly to the mucosal surface of the intestine. Consequently, a LAB-based drug delivery system (DDS) can have synergistic effects: the patient derives benefit from both the LAB and the therapeutic cargo. Furthermore, the localized effect of LAB-based DDS in the intestine would ensure targeted treatment at the site of disease; this has the benefits of requiring a lower dose of a drug, with fewer systemic side effects. In this review, we discuss the evidence supporting the beneficial effects of LAB-based DDS and its application to CRC.

Introduction

Benefits of LAB

Lactic acid bacteria (LAB) and other probiotic bacteria are living supplementary organisms that are beneficial to the host; in addition, these bacteria are safe, with a history of use as a foodstuff going back thousands of years [1,2]. Two major advantages are their proven overall health benefits to the host and their potential as a vehicle for therapy [3]. Many studies have examined the role of the human gut microbiota; indeed, colonization of the gut by LAB provides balance and contributes to the health of the host [4]. A review by An *et al.* discusses recent insights into the cellular and molecular mechanisms underlying the beneficial effects of LAB; these mechanisms are related to cell cycle arrest, apoptosis, immune responses, inflammatory responses, antioxidant DNA damage, and epigenetics [5].

Potential of LAB as an anti-cancer therapeutic

As well as providing health benefits in their own right, LAB can also play a role in therapy [3]. Indeed, animal and preliminary human studies demonstrate the therapeutic effects of LAB in the context of various diseases, including cancer [5,6]. Recent studies show that LAB suppress growth of colon, stomach, breast, cervix, and myeloid leukemia cells via multiple pathways [7,8]. Thus, LAB-based regimens are often used as an adjuvant during anti-cancer chemotherapy. However, because orally administered LAB reside in the gastrointestinal tract and colonize the host intestine, they are likely to be most useful as therapeutics for gastrointestinal cancers; indeed, numerous animal studies show the positive anti-cancer effects of LAB [5,9,10]. Colorectal cancer (CRC), which begins as a polyp, is the most common malignancy of the gastrointestinal tract and remains one of the most common causes of death worldwide [11]. However, LAB show promise as a means of preventing and suppressing progression of CRC [12]. Moreover, oral administration of LAB normalizes the balance within the intestinal

microflora, improves the gastrointestinal barrier, inhibits the growth of potential pathogens, and suppresses carcinogenesis in the gut [5].

Genetic engineering of LAB

Recent studies raise the possibility of using LAB as bio-therapeutic agents to develop cutting-edge cancer therapies. Genetic engineering methods have allowed modification of LAB, particularly *Lactococcus lactis* (*L. lactis*) and others, for industrial or medical purposes [13]. Gene expression systems have been developed to facilitate genetic engineering of LAB, thereby allowing controlled expression of target genes using various promoters that are regulated by sugar (the lactose operon promoter) [14] and salt (the *gadC* promoter) [15] concentrations, temperature upshifts (the *tec* phage promoter) [16], reduced pH (P170) [17], or phage infection (the *phi31* promoter) [18]. Although promoter-inducible systems are not always easy to regulate, introduction of a well-characterized promoter might allow controlled expression of downstream genes and could be applied to diverse LAB species [19]. Future innovations regarding the therapeutic use of LAB are necessary to validate these improvements and establish reliable and safe therapeutic strategies. These challenges could lead to a medical breakthrough in the fields of immunotherapy and cancer treatment, as well as improve delivery of anti-inflammatory drugs to treat other bowel diseases.

Below, we discuss the genetic modification of LAB and their use as bio-therapeutic agents for cancer therapy. An important property

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of LAB is that they are regarded as “food-grade” because of their safe application as probiotics when compare with other bacteria.

Plasmid vector systems

Plasmids, diverse expression systems that harbor selective markers, allow expression and maintenance of exogenous molecules in the host. Plasmids for LAB contain a replication source from a natural plasmid combined with a food-grade selection marker [20-23]. Food-grade selection markers for LAB are divided into two groups: selectable markers that confer a new phenotype (such as sugar utilization or bacteriocin resistance) [24], and complementation markers (e.g., alanine racemase [25] and thymidylate synthase [26]) that restore an impaired function to promote cell viability. Although plasmid vector systems are versatile, they have several limitations, including copy number alteration, loss of selection markers, and poor stability [27].

Chromosome modification systems

Allelic replacement in the chromosome is a natural phenomenon that allows genetic modification or insertion of stable DNA into host chromosomes without leaving behind unnecessary foreign DNA. Allelic replacement occurs via double crossover between two homologous regions that flank the modification and the corresponding region on the chromosome. This natural form of genetic transformation is common in *Streptococcus pneumoniae* (*S. pneumoniae*) [28] and *Bacillus subtilis* (*B. subtilis*) [29]. For example, direct homologous integration of a thermosensitive plasmid into the *L. lactis* chromosome carrying a chromosomal DNA fragment (≥ 500 bp) was achieved by sequential recombination of two fragment flanking modifications, such as nucleotide changes and DNA deletions or insertions [30]. This thermosensitive plasmid vector might be cured during several generations at 37°C; therefore, it might be useful for selecting food-grade mutants containing a single insertion element (e.g., a new DNA fragment) in the genome [31].

Therapeutic applications

Whenever food is ingested, the gastrointestinal tract is exposed to environmental microbes (including probiotics or pathogens). Diseases of the gastrointestinal tract may be caused by invasion of harmful bacteria or pathogens. However, colonization of the human intestine by LAB has probiotic effects that benefit health by improving the balance between “good” and “bad” bacteria in the intestine [32]. Interestingly, some LAB strains may also be adapted to act as therapeutic microorganisms [33]. There are many diseases of the gastrointestinal tract; among these, the global incidence of CRC is continuing to increase [34,35]. Chemotherapeutic regimens are used routinely to treat advanced-stage colon cancer; however, the response rates are poor, and the treatments have adverse side effects [35,36]. Therefore, novel therapeutic or preventive agents with minimal or no side effects are required urgently. LAB are potential novel therapeutics that can be used to treat CRC; the location of these bacteria places them in a prime position to target CRC, thereby directly or indirectly preventing or treating the disease [37-39]. Many studies have used genetically modified LAB to improve their therapeutic capacity against CRC. For example, Cell Biotech have developed a genetically modified strain of *Pediococcus pentosaceus*, SL4 (*P. pentosaceus* SL4), which secretes the LAB-derived anti-cancer protein p8; a previous study demonstrates significant suppression of CRC after oral administration to a mouse xenograft model [40]. In addition, we successfully constructed a mammalian expression vector harboring the p8 gene for use as a vehicle for gene therapy [41]. The advantage of these approaches is that LAB-derived proteins are both beneficial and safe

for humans [42]. A study by de Moreno de LeBlanc *et al.* suggests that oral administration of a catalase-producing *Lactococcus lactis* (*L. lactis*) protects mice from chemically induced CRC [43], whereas another study shows that localized delivery of therapeutic doses of interleukin (IL)-10 by genetically engineered *L. lactis* results in a 50% reduction in the incidence of colitis in mice, suggesting a potential treatment for inflammatory bowel disease [44]. In addition, Steidler *et al.* reported improved delivery of IL-10 via *L. lactis* using a bio-containment system based on genomic integration, in which the *IL-10* gene was replaced by the thymidylate synthase gene (*thyA*) [45]. Increasing evidence suggests that genetic modification of LAB improves their health-promoting properties and their ability to protect against gastrointestinal diseases; furthermore, genetic modification means that LAB can be targeted to specific diseases with few, if any, toxic side effects.

Conclusions

Current cancer therapies have limited efficacy because they are highly toxic to both cancer cells and normal tissue. A growing body of evidence suggests that LAB have chemopreventive effects, even though the magnitude of these effects (therapeutic activity) does not match that of chemical drugs. However, LAB can be used as a natural adjuvant for chemotherapy, and they can be engineered to deliver therapeutic payloads in a targeted manner. Many creative approaches have been used to exploit natural bacterial processes and/or to harness bacteria as therapy vectors and cancer cell destroyers. Some of the safety concerns associated with genetic engineering need to be overcome; however, we believe that LAB are an important new weapon in the fight against cancer and other immune diseases.

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Author contributions

Byung Chull An: Organizing and manuscript writing.

Yongku Ryu: Benefits of probiotics.

Oksik Choi: Application of DDS on LAB.

Sunwoong Hong: Anti-cancer activity of LAB.

Jin Young Heo: Genetic engineering of LAB.

Myung Jun Chung: Supervisor of this study.

Competing interest

The authors have no conflict of interests to declare.

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