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ORIGINAL ARTICLE

Heterologous expression of carcinoembryonic antigen in *Lactococcus lactis* via LcsB-mediated surface displaying system for oral vaccine development

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Background/Purpose: Carcinoembryonic antigen (CEA) is an attractive target for immunotherapy because it is expressed minimally in normal tissue, but is overexpressed in a wide variety of malignant epithelial tissues. Lactic acid bacteria (LABs), widely used in food processes, are attractive candidates for oral vaccination. Thus, we examined whether LABs could be used as a live vaccine vector to deliver CEA antigen.
Methods: CEA was cloned into an *Escherichia coli/Lactococcus lactis* shuttle vector pSEC:LEISS under the control of a nisin promoter. For displaying the CEA on the cell surface of the *L. lactis* strain, the anchor motif LcsB from the S-layer protein of *Lactobacillus crispatus* was fused with CEA. Intracellular and cell surface expression of the CEA–LcsB fusion was confirmed by western blot analysis.
Results: Significantly higher levels of CEA-specific secretory immunoglobulin A in the sera of mice were observed upon oral administration of strain cultures containing the CEA–LcsB fused protein. In addition, the CEA–LcsB antigen group showed a higher spleen index compared to the CEA antigen alone or negative control, demonstrating that surface-displayed CEA antigen could induce a higher immune response.

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Conclusion: These results provided the first evidence for displaying CEA antigen on the cell surfaces of LABs as oral vaccines against cancer or infectious diseases.

Introduction

Carcinoembryonic antigen (CEA), which was first identified in human colon cancer in 1963, is a 180-kDa glycoprotein. Normally, CEA can be expressed in limited areas of the adult human body, but it is overexpressed in a high percentage of adenocarcinomas, particularly in those of the colon, pancreas, breast, lungs, rectum, and stomach. Hence, CEA is used as a tumor-related marker at clinic diagnosis.[1,2] Previous studies have proved that CEA has high immunogenicity and can elicit strong T-cell and humoral immune responses, which are helpful in inhibiting the growth of malignant tumors *in vivo*.[3] Apparently, development of a CEA-based vaccine to induce potent T-cell immunity will be significant in the immunotherapy of tumors.

Many strategies for development of CEA-based vaccines have been reported, including recombinant live vector vaccines (viral and/or bacterial vector vaccines), nucleic acid vaccines (DNA and/or RNA replicon vaccines), and protein or peptide vaccines.[4–8] Viral vectors or DNA vaccines offer the ability to deliver antigen into the cytosol, and therefore into the conventional major histocompatibility complex (MHC) class I processing pathway. However, concern about the disadvantages, including the limited DNA carrying capacity, toxicity, immunogenicity, the possibility of random integration of the vector DNA into the host genome, and their high coat, may limit the application of viral and DNA vaccines.[9,10] Thus, to circumvent these issues, the use of live, food-grade, noninvasive, nonpathogenic bacteria as antigen vehicles is a promising strategy.

Lactic acid bacteria (LABs) are generally regarded as safe microorganisms, and some of them are able to stimulate the immune system of the host as adjuvants due to their probiotic properties and their immunomodulation capacity, which makes them advantageous live vaccines.[11,12] Among LABs, *Lactococcus lactis* is, by far, the most extensively studied with respect to its physiology, metabolic pathways, and regulatory mechanisms, and its genome was the first LAB genome to be completely sequenced and annotated.[13] Because of its ability to pass through the intestinal tract without colonization, the use of *L. lactis* as a vaccine vector is emerging as one of the most advanced prototypes of a possible new class of bacterial vaccines derived from noninvasive, nonpathogenic Gram-positive bacteria.[11,14,15] Immunization with *L. lactis*-delivered vaccines elicits immune responses specific to heterologous antigens.[16,17] To date, many expression systems have been developed to produce recombinant proteins for various biotechnological applications in LABs. The best-characterized controllable expression system is based on the use of the *nisin biosysthesis* promoter p*nisA*. This system is versatile and can be used to produce large quantities of prokaryotic and eukaryotic proteins because there is a linear dependency between the amount of nisin added to the culture medium and the promoter activity.[18] Several antigens (e.g., L7/L12, TTFC, HA, and capsid protein of PCV) have been successfully expressed using the NICE system (nisin-controlled gene expression) or its derivatives.[16,17,19,20] Note that a protective immune response depends not only on the antigen and the delivery vehicle, but also on the location of the antigen.[19] Various genetic engineering tools have been developed to express antigens and therapeutic molecules efficiently at different cellular localizations (i.e., cytoplasm, cell wall, or extracellular medium).[19,21] To display antigen on the cell surface of *L. lactis* cells, various anchoring motifs, such as LysM and LcsB, as well as M6 protein, have also been explored for their efficiency in attaching hybrid protein to the cell membrane or cell wall of LABs.[19,22–24]

In this work, we constructed genetically modified *L. lactis* that produced recombinant CEA antigen by using the NICE system. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting showed that CEA was displayed on the cell surface of *L. lactis* under the aid of the anchoring motif LcsB. Immunization analysis demonstrated that the surface-displayed CEA induced an immune response according to activation of the spleen and secretion of immunoglobulin A (IgA).

Methods

Bacterial strains and growth conditions

All of the strains, plasmids, and oligonucleotide primers used in this study are described in Table 1. *L. lactis* NZ9000 was grown in M17 broth (Oxoid, Basingstoke, Hants, UK) supplemented with 0.5% (w/v) glucose (GM17) at 30°C or 42°C anaerobically, respectively. *Escherichia coli* DH5α was grown aerobically in Luria–Bertani broth at 37°C. Chloramphenicol was used at 10 μg/mL and 5 μg/mL for *E. coli* and *L. lactis*, respectively.

BALB/c mice (6–8 weeks old; Medical Experimental Center of Shandong University, China) were housed in a pathogen-free isolator under sterile conditions in the animal facilities of Jinan Health Science Exchange and Service Center (China). All experiments were performed according to protocols in accordance with institutional guidelines.

Plasmids and DNA manipulations

Standard recombinant DNA techniques were performed as described previously.[23] Electroporation of *L. lactis* was performed using a Gene Pulser (2500 V, 200 Ω, 25 μF, 5 ms,