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COMMENTARY

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Antitumor effect of oral cancer vaccine with *Bifidobacterium* delivering WT1 protein to gut immune system is superior to WT1 peptide vaccine

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ABSTRACT

Despite the revolutionary progress of immune checkpoint inhibitors (CPIs) for cancer immunotherapy, CPIs are effective only in a subset of patients. Combining CPIs and cancer vaccines to achieve better clinical outcomes is a reasonable approach since CPI enhances cancer vaccine-induced tumor-associated antigen (TAA) specific CTL. Among the various TAAs so far identified, WT1 protein is one of the most promising TAAs as a cancer vaccine target. Until now clinical trials of WT1 vaccine have demonstrated only modest clinical efficacy. These WT1 vaccines were based on peptides or dendritic cells (DCs), and there was no oral cancer vaccine. Recently, we developed a WT1 oral cancer vaccine using a recombinant *Bifidobacterium* displaying WT1 protein, which can efficiently deliver WT1 protein to the gut immune system, and we demonstrated that this oral cancer vaccine had a significant anti-tumor effect in a C1498-WT1 murine leukemia syngeneic tumor model. The WT1 protein displayed in this vaccine consists of about 70% of the WT1 amino acid sequence including multiple known CD4 and CD8 T-cell epitopes of WT1. In this commentary, we introduce our recent data indicating the superior anti-tumor effect of a WT1 oral cancer vaccine delivering WT1 protein to the gut immune system compared to a peptide vaccine.

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Commentary

Background

Cancer immunotherapy has entered the standards of cancer care with the development of immune checkpoint inhibitors (CPI) such as PD-1/PD-L1 and CTLA-4 inhibitors.[1] However, CPIs remain effective for the inhibition of immunosuppressive signals toward cancer cells only in a subset of patients.[2,3] An approach combining CPIs and tumor immunostimulatory therapy such as cancer vaccines could reasonably be expected to increase the response rate of CPIs and achieve better clinical outcomes.[4] Cancer vaccines that forcibly induce a tumor-associated antigen (TAA)-specific T cell response can be enhanced by combination with CPIs.[5] To date, various TAAs have been identified as cancer vaccine targets.[6]

In 2009, a National Cancer Institute pilot project developed a priority-ranked list of cancer vaccine target antigens based on predefined and preweighted objective criteria including therapeutic function, immunogenicity, oncogenicity, specificity, expression level and percent positive cells, among others. In that list, Wilms' tumor 1 (WT1) protein was ranked as the No. 1 antigen among 75 selected TAAs.[7] Despite the high potential of WT1 as a cancer vaccine target antigen, most clinical trials (phase I to II) of WT1 vaccines demonstrated only antigen-specific immune response, not significant clinical efficacy.[8] These findings suggest that another technical innovation is required for the practical application of WT1 vaccine to treat cancer patients.

Previous WT1 vaccines used in the clinical trials were based on peptides or dendritic cells (DCs). There was no oral cancer vaccine. We have developed an oral vaccine platform using *Bifidobacterium*, which can efficiently deliver antigen protein to the gut immune system, and demonstrated that this oral vaccine platform could induce both humoral and cellular strong immunity.[9,10] We recently constructed a WT1 oral cancer vaccine (*B. longum* 420) displaying murine WT1 protein on the cell surface of *Bifidobacterium longum*.[11] The WT1 protein displayed in this vaccine consists of about 70% of the WT1 amino acid sequence including multiple known CD4 and CD8 T-cell epitopes of WT1.[12] In our previous study, we demonstrated that oral administration of *B. longum* 420 induced a significant *in vivo* anti-tumor effect compared to *B. longum* 2012, which is a recombinant *Bifidobacterium longum* transfected with shuttle vector not containing the WT1 protein, in a syngeneic mouse tumor model using C1498-WT1 cells, C57BL/6 origin recombinant murine leukemia cells stably expressing murine WT1 protein.[11]

This WT1 oral cancer vaccine has most of the WT1 protein length containing multiple known CD4 and CD8 T-cell epitopes of WT1 and is functionally able to utilize the gut immune system; therefore, we hypothesized that it should have a superior anti-tumor effect to previous peptide vaccines. Here we compared the antitumor effects of our WT1 oral cancer vaccine versus a WT1 peptide vaccine, Db126,[12] in a syngeneic mouse tumor model using TRAMP-C2, murine prostate cancer cells naturally expressing WT1 protein.[13]

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Experimental design

The details of the WT1 oral cancer vaccine, a recombinant *B. longum* 420 displaying a partial murine-WT1 protein (117–419 amino acid residues), and a recombinant *B. longum* 2012 displaying only a GLBP protein were described in our previous paper.[11] An MHC class I (H-2D^b)-binding peptide, Db126 peptide vaccine (a.a.126–134 RMFPNAPYL),[12] was obtained from Eurofins Genomics (Tokyo, Japan). We compared anti-tumor effects of our WT1 oral cancer vaccine[11] vs the Db126 peptide vaccine[14] using a TRAMP-C2 murine prostate cancer cell syngeneic mouse tumor model.[13] In addition, the tetramer assay using H-2D^b WT1 Tetramer-RMFPNAPYL (MBL Co., Ltd, Nagoya, Japan) was performed to examine whether the WT1 oral cancer vaccine and the Db126 peptide vaccine could induce the WT1 epitope-RMFPNAPYL specific CTLs or not, with the same method as our previous study.[11] All aspects of the experimental design and procedure were reviewed and approved by the institutional ethics and animal welfare committees of Kobe University.

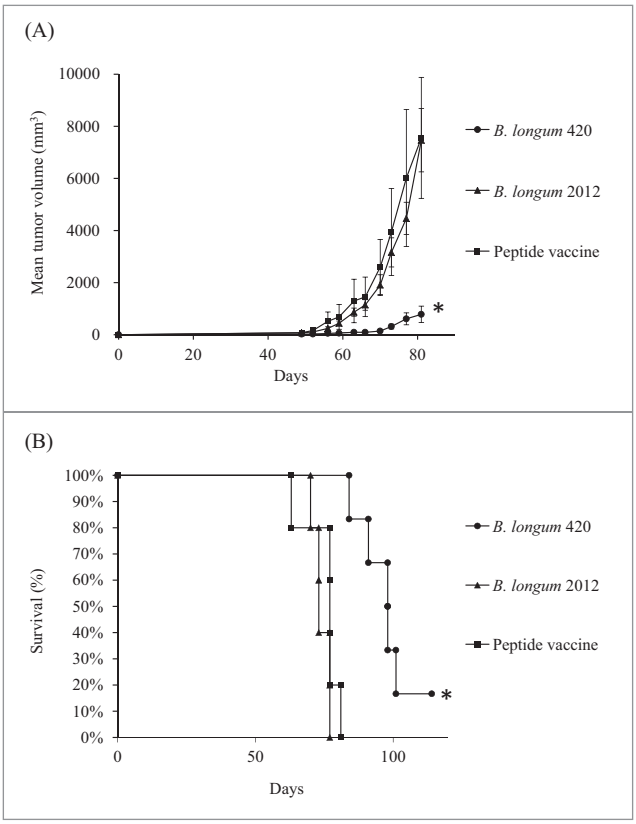

Figure 1. Anti-tumor effect of *B. longum* 420 oral vaccine vs Db126 peptide vaccine. Male C57BL/6 mice (H-2D^b, n = 6 per group) orally received with 1×10^9 colony forming units of *longum* 2012 or *B. longum* 420 for 5 days a week for 2 weeks, or itraperitoneally received 100 μg of Db126 peptide vaccine emulsified with incomplete Freund's adjuvant (Wako Pure Chemical, Osaka, Japan) for once a week for 2 weeks. After the 2 weeks of vaccination, 5×10^5 TRAMP-C2 cells were injected into the right flank of the mice. Subsequently, vaccination was carried out for the next 4 weeks. Booster vaccinations were conducted in the 8th and 9th weeks. Tumor volume was monitored after tumor inoculation. Mice were euthanized when tumor diameter was >20 mm. (A) Tumor growth curves of mice with TRAMP-C2 tumor ($^*p<0.05$). Each data point represents the average of each group (bars, ±SE). (B) Kaplan-Meier survival curve of mice with TRAMP-C2 tumor ($^*p<0.05$).

B. longum 420 demonstrated a marked anti-tumor effect, while Db126 peptide vaccine did not show any anti-tumor effect

In the animal study, *B. longum* 420 markedly inhibited tumor growth compared with Db126 peptide vaccine and *B. longum* 2012 (Fig. 1A). At 81 days after the tumor inoculation, the mean tumor volume in the *B. longum* 420 group was significantly smaller than in the other groups ($p<0.05$). In addition, *B. longum* 420 significantly prolonged the survival of mice bearing TRAMP-C2 tumors compared with other treatment groups ($p < 0.05$) (Fig. 1B). In contrast, peptide vaccine did not show any anti-tumor effect or improvement of survival. Interestingly, compared to our previous *in vivo* data using a C1498-WT1 syngeneic tumor model,[11] we observed a more remarkable tumor growth inhibitory effect in this TRAMP-C2 syngeneic tumor model. This may be due to the fact that the TRAMP-C2 is a murine prostate cancer cell line naturally expressing WT1 protein,[13] and the C1498-WT1 is a murine leukemia cell line stably transfected with murine *WT1* gene.[12]

Both B. longum 420 and Db126 peptide vaccines could induce RMFPNAPYL-specific CTL

To investigate the induction of WT1 CD8 (MHC class I) T-cell epitope RMFPNAPYL -specific CTLs, we performed a H-2D^b-WT1 RMFPNAPYL-tetramer assay using immunized splenocytes. The Db126 peptide of a.a.126-134: RMFPNAPYL is one of the best-known WT1 CD8 T-cell epitopes and homologous to human HLA-A*0201 restricted WT1 (a.a.126-134) CD8 T-cell epitope.[15] As a result, the frequency of CD8 T cells responding to the H-2D^b-restricted WT1 epitope (RMFPNAPYL) significantly increased in *B. longum* 420-immunized splenocytes compared with the other groups when splenocytes were stimulated with TRAMP-C2 cell lysate ($^*p < 0.01$, $^{**}p < 0.05$) (Fig 2A), and in Db126 peptide -immunized splenocytes compared to the B. *longum* 2012 group when splenocytes were stimulated with Db126 peptide (Fig. 2B). These results suggested that although the Db126 peptide vaccine certainly induced Db126 peptide-specific CTLs, it failed to induce a substantial anti-tumor effect.

Bacterial vector for vaccine and cancer therapy

Our WT1 oral cancer vaccine was developed using the *Bifidobacterium* bacterial vector. Ty21a, a chemical mutant of *Salmonella enterica* serovar Typhi (*S.* Typhi), was originally used as an oral Typhoid vaccine.[16] With the rapid progress of gene engineering, *Salmonella* spp. are currently being used as an oral vaccine platform against several infectious and cancerous diseases because of their natural tropism to gut associated lymphoid tissues (GALT) thorough Microfold (M) cells.[17,18] In animal experiments, a *Salmonella* mutant expressing *Mycobacterium tuberculosis* fusion antigen Ag85B-ESAT6 demonstrated substantial vaccine efficacy as an oral Tuberculosis vaccine.[19] Other Live Attenuated pathogenic bacteria, such as *Listeria monocytogenes*[20] and *Vibrio cholerae*[21] are also being used as a vector to deliver vaccine target antigens, antigen genes, or therapeutic anti-cancer agents.[17] Currently probiotic bacteria

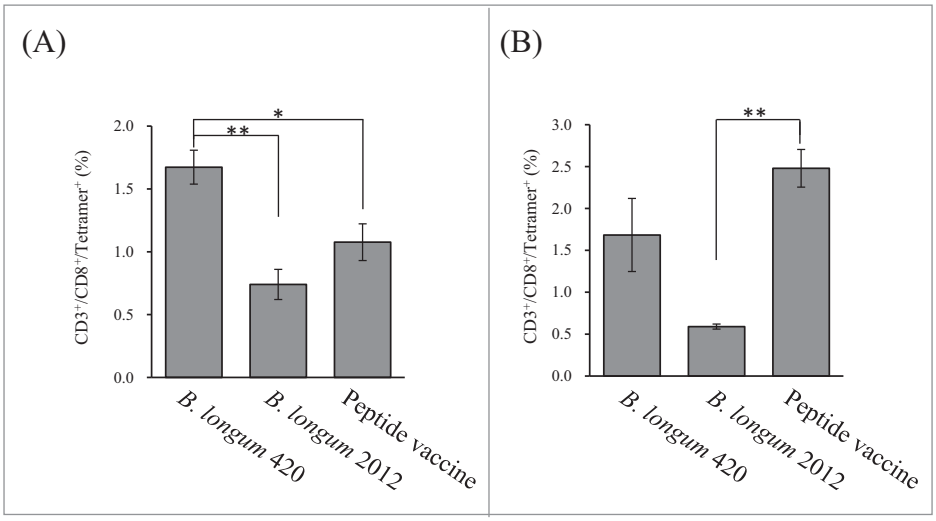

Figure 2. Detection of WT1 (RMPNAPYL)-specific CTLs by tetramer assay. After the last vaccination of the 6 consecutive weeks, splenocytes in the three treatment groups (*B. longum* 2012, *B. longum* 420, and Db126 peptide; n = 3 per group) were isolated and re-stimulated with mitomycin C-treated TRAMP-C2 cells (A) or WT1 RMPNAPYL-peptide (B) in the presence of murine IL-2 for 7 days *in vitro*. The frequency of WT1 (RMPNAPYL) T-cell epitope-specific CD8T cells was determined by H-2D^b tetramer (* $p<0.05$, ** $p<0.01$).

including *Lactobacillus* and *Bifidobacterium* are being investigated as non-pathogenic and safer oral vaccine platforms.[17] Oral vaccination with an attenuated *Lactobacillus casei* expressing human papilloma virus (HPV) E7 protein succeeded in inducing antigen-specific cellular immunity in patients with cervical intraepithelial neoplasia (CIN).[22] Previously, Hiramatsu et al. reported that when *Bifidobacterium* was orally given to mice, *Bifidobacterium* was detected in Peyer's patch within one hour and appeared with dendritic cells in mesenteric lymph node (MNL) after 20 hours.[23] Based on this natural tropism of *Bifidobacterium*, our WT1 cancer vaccine could deliver WT1 protein into DCs in Peyer's patch. Then DCs loaded with the WT1 protein could move to the MNL where the DCs presenting properly processed WT1 peptides interact with T lymphocytes. Indeed, we confirmed that oral vaccination with this WT1 vaccine could induce WT1 epitope (RMFPNAPYL)-specific CTL in mice.[11]

Clinical trials of WT1 cancer vaccine

Currently, at least 27 clinical trials for WT1 cancer vaccine have been registered to ClinicalTrials.gov, a service of the U.S. National Institutes of Health.[24] In detail 15 of these are trials of WT1 peptide vaccines, 11 are trials of DC-based vaccine, and one is a trial of WT1 peptide DNA vaccine.[25] The HLA-A*0201-binding WT1(126-134) peptide[26] is frequently used for clinical trials of WT1 peptide vaccine and most peptide vaccines are administered with Montanide or Freund's adjuvants. In our animal experiment, oral WT1 vaccine demonstrated better anti-tumor effects without adjuvant than WT1 (126–134: RMFPNAPYL) Db126 peptide vaccine with Freund's adjuvant in the TRAMP-C2 mouse syngeneic tumor model. Although the Db126 peptide vaccine could certainly induce the RMFPNAPYL-specific CTL, it did not achieve any antitumor effect. This result supports our hypothesis that a cancer vaccine containing multiple CD4 and CD8 T-cell epitopes has superior anti-tumor effects over a single peptide vaccine. Further studies are warranted to confirm the clinical feasibility of our oral WT1 cancer vaccine.

Conclusion

In conclusion, we developed an oral cancer vaccine consisting of a recombinant *Bifidobacterium* displaying WT1 protein including multiple CD4 and CD8 T-cell epitopes and utilizing the gut immune system, and confirmed its superior anti-tumor effect over the WT1 peptide vaccine. Besides the great practical advantages of an oral preparation, this WT1 oral cancer vaccine also possesses greater potential efficacy.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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