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Brief Communication

Anti-tumor Effect of 4-1BBL Modified Tumor Cells as Preventive and Therapeutic Vaccine

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We have previously reported that genetically modified tumor cells with 4-1BBL have anti-cancer effects in a CT26 mouse colorectal tumor model. In this study, genetically modified tumor cells with 4-1BBL were evaluated for their potential as candidates for preventive and therapeutic cancer vaccine. To identify the effect of preventive and therapeutic vaccine of genetically modified tumor cells with 4-1BBL, tumor growth pattern of CT26-4-1BBL as a cancer vaccine was examined compared to CT26-beta-gal. In therapeutic vaccination, CT26-WT was inoculated into mice and then vaccinated mice with doxorubicin (Dox)-treated CT26-beta-gal and CT26-4-1BBL (single or three times). Triple vaccination with Dox-treated tumor cell inhibited tumor growth compared to single vaccination. Vaccination with CT26-4-1BBL showed an efficient tumor growth inhibition compared to vaccination with CT26-beta-gal. For preventive vaccination, Dox-treated CT26-beta-gal and CT26-4-1BBL was vaccinated into mice with three times and then administered mice with CT26-WT. Preventive vaccination with CT26-4-1BBL showed no tumor growth. Preventive vaccination with CT26-beta-gal also led to tumor-free mice. These results suggest that genetically modified tumor cells with 4-1BBL can be used as therapeutic or preventive cancer vaccine.

Key Words: 4-1BBL, Therapeutic vaccine, Preventive vaccine, Tumor growth inhibition

Cancer immunotherapy has many limitations. For example, cancer cells can avoid immune recognition (Kim and Cho, 2022; Vinay et al., 2015). In addition, immunosuppression of the tumor microenvironment makes tumor elimination ineffective (Munn and Bronte, 2016; Tang et al., 2021). Despite these limitations, considerable progress has been achieved in the field of therapeutic and preventive cancer immunotherapy (Hollingsworth and Jansen, 2019; Kantoff et al., 2010; Kooreman et al., 2018; Lipson et al., 2015; Srivatsan et al., 2014). Cancer vaccines can utilize tumor-associated antigens and tumor-specific antigens to activate immune system and induce both cellular immunity and humoral immune response to inhibit tumor growth and eradicate cancer cells. There are several cancer vaccine platforms, including cell-based vaccines (Jin and Wang, 2021; Santos and Butterfield, 2018), peptide-based vaccines (Schneble et al., 2016), viral-based vaccines (Larocca and Schlom, 2011), and nucleic acid-based vaccines (Lopes et al., 2019). Among them, whole tumor cell-based vaccine is useful for obtaining a broad range of tumor-associated antigen or tumor-specific antigen for cytotoxic CD8 T cell activation (Sadeghi Najafabadi et al., 2022). Dead tumor cells can induce adaptive immune response (Ullrich et al., 2008). However, dead tumor cells alone are not very effective as a

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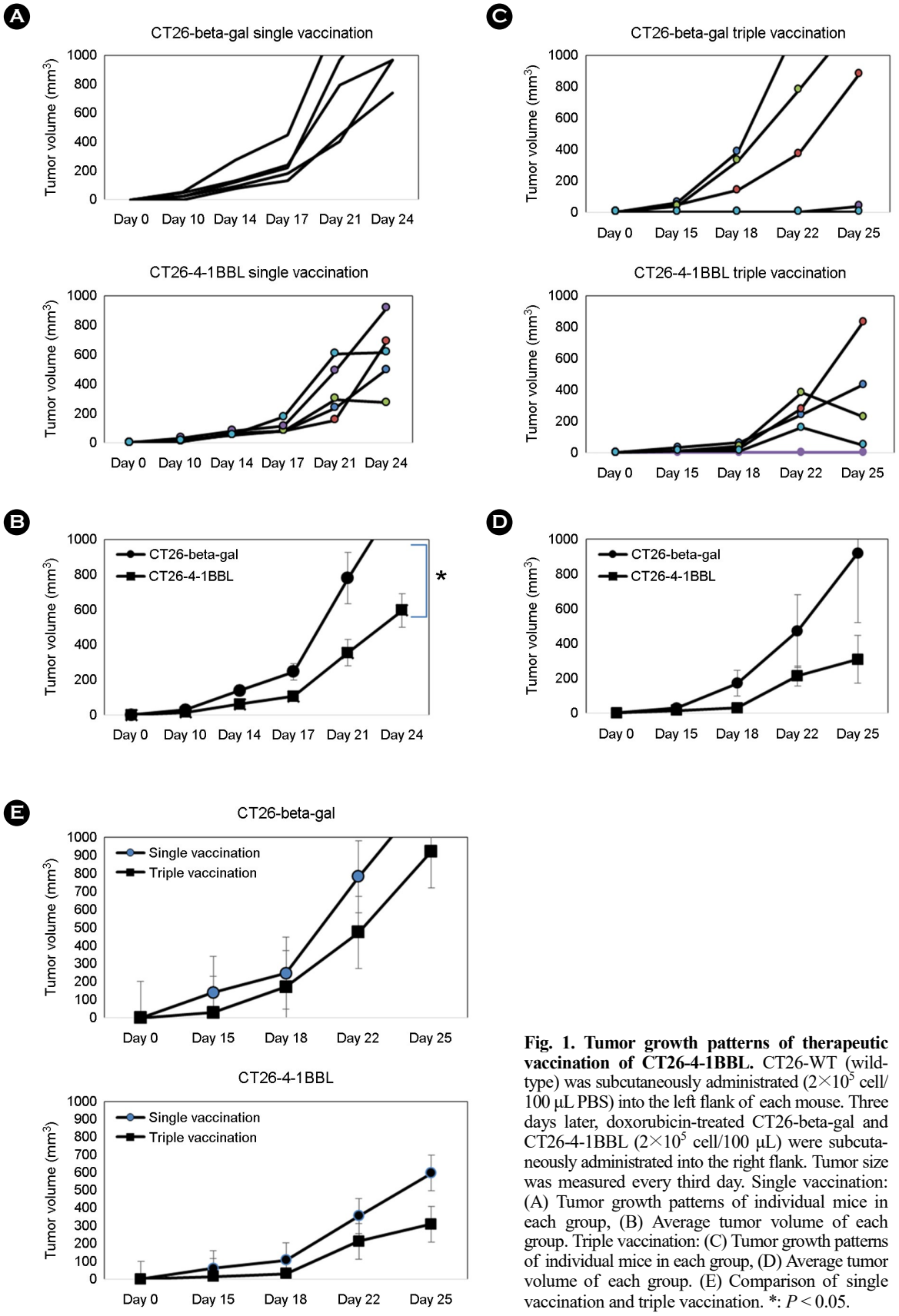

Fig. 1. Tumor growth patterns of therapeutic vaccination of CT26-4-1BBL. CT26-WT (wild-type) was subcutaneously administrated (2×10^5 cell/100 μL PBS) into the left flank of each mouse. Three days later, doxorubicin-treated CT26-beta-gal and CT26-4-1BBL (2×10^5 cell/100 μL) were subcutaneously administrated into the right flank. Tumor size was measured every third day. Single vaccination: (A) Tumor growth patterns of individual mice in each group, (B) Average tumor volume of each group. Triple vaccination: (C) Tumor growth patterns of individual mice in each group, (D) Average tumor volume of each group. (E) Comparison of single vaccination and triple vaccination. *: $P < 0.05$.

vaccine (Jin and Wang, 2021). Modification of tumor cells could improve the efficacy of whole tumor cell vaccine. Genetically modified whole tumor cell strategies have been established using several immune-regulatory molecules such as interleukin-2 (Rosenberg, 2014), interferon α (Sartoris et al., 2011), granulocyte-macrophage colony-stimulating factor (Eager and Nemunaitis, 2005), and co-stimulatory molecule (Douin-Echinard et al., 2000) as adjuvants. We have previously reported that 4-1BBL costimulatory molecule genetically modified tumor cell has an anti-tumor effect through cytotoxic CD8 T cells (Kim, 2019; Kim, 2021).

In this study, we hypothesized that genetically modified tumor cells with 4-1BBL could be used as a therapeutic and preventive vaccine. To test this hypothesis, we analyzed tumor growth patterns of CT26 colorectal cancer cells.

Six to 8-week-old *Balb/c* female mice were purchased from OrientBio (Korea). These mice were bred under pathogen-free conditions and maintained by approved institutional animal care protocols. CT26 colorectal cancer cells were purchased from ATCC (the American Type Culture Collection, Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium (DMEM) with 10 mM L-glutamine, 0.1% gentamicin, 100 U/mL penicillin/streptomycin, and 10% fetal bovine serum (FBS). Doxorubicin was purchased from Sigma (MO, USA). In therapeutic vaccination experiment, CT26-WT (wildtype) (2×10^5 cells/100 μL PBS) was subcutaneously administrated into the left flank of each *Balb/c* mouse. After 3 days, CT26-beta-gal and CT26-4-1BBL (2×10^5 cells/100 μL) cells treated with doxorubicin at 25 μM overnight were subcutaneously administered into the right flank of each mouse. In preventive vaccination experiment, before subcutaneous implantation of CT26-WT tumor cells (2×10^5 cells/100 μL PBS) into the left flank of each *Balb/c* mouse, doxorubicin-treated CT26-beta-gal and CT26-4-1BBL cells were used for vaccination three times every three days. Tumor size was gauged in two dimension using calipers and tumor volume was calculated as follows: tumor area (mm^3) = length × width^2. Data are presented as the means ± SEM (standard error of mean). Significance of differences among tumor growth patterns of each group was determined using two-tailed Student's *t*-test and $P < 0.05$ were considered significant. To determine the effect of vaccination of genetically modified tumor cells, we examined tumor growth patterns of CT26-WT after therapeutic and preventive vaccination using CT26-beta-gal and CT26-4-1BBL. After single therapeutic vaccination, tumor growth patterns of CT26-WT in mice vaccinated with doxorubicin-treated CT26-beta-gal and CT26-4-1BBL were measured. Single vaccination with CT26-4-1BBL was significantly superior to that of CT26-beta-gal on day 24 (Figs. 1A and 1B). There were two tumor regressed individual mice after single vaccination of CT26-4-1BBL (Fig. 1A, right panel). After triple therapeutic vaccination, both CT26-beta-gal and CT26-4-1BBL groups showed more inhibition of tumor growth compared to single vaccination of each group. Triple vaccination with CT26-4-1BBL also inhibited tumor growth compared to CT26-beta-gal, although such inhibition was not statistically significant (Fig. 1D). There was one tumor-free mouse in each group after triple vaccination. There

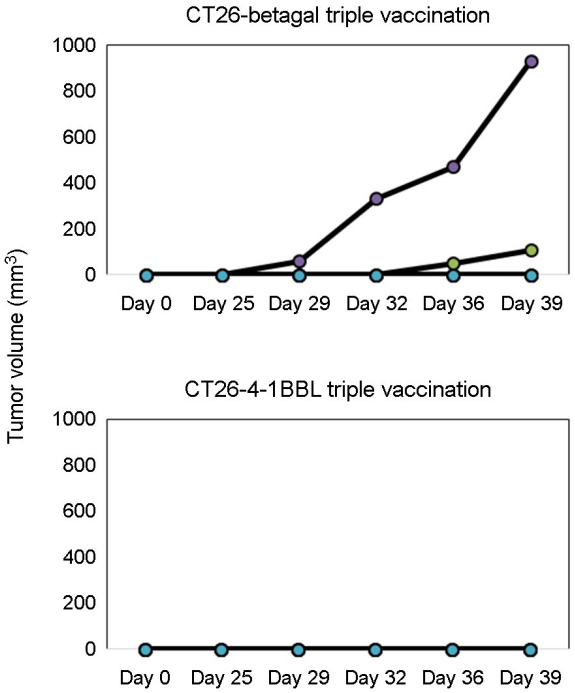

Fig. 2. Tumor growth patterns of preventive vaccination of CT26-4-1BBL. Before subcutaneous implantation of CT26-WT tumor cells (2×10^5 cell/100 μL PBS) into the left flank of each *Balb/c* mouse, doxorubicin-treated CT26-beta-gal and CT26-4-1BBL cells were used to vaccinate mouse three times every third days. Three days after the last tumor cell vaccination, CT26-WT tumor cells were administered. Tumor size was measured every third day.

were two tumor-regressed individual mice in after CT26-4-1BBL vaccination (Fig. 1C). Triple vaccination of CT26-4-1BBL reduced tumor growth compared to single vaccination of CT26-4-1BBL (Fig. 1E). These data showed that genetically modified tumor cells with 4-1BBL could be used as therapeutic cancer vaccine and that the number vaccination would be a major factor to be considered as cancer vaccine. In preventive vaccination, triple vaccination with doxorubicin -treated CT26-beta-gal or CT26-4-1BBL was conducted before CT26-WT tumor cell administration. There were three tumor-free mice after CD26-beta-gal preventive vaccination. It means that allogenic dead tumor cells could induce anti-tumor effect through immune response. After CT26-4-1BBL preventive vaccination, all individual mice had no tumor growth (Fig. 2). It means that 4-1BBL could act as a potent immune-stimulant after preventive vaccination. In this study, we analyzed tumor growth patterns of genetically modified tumor cells with 4-1BBL through preventive and therapeutic ways and showed the possibility of developing cancer vaccine using genetically modified tumor cells with 4-BBL. In the future, we will investigate the vaccine effect of genetically modified tumor cell with additional gene and identify the immune mechanism involved in the effect of cancer vaccine.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Douin-Echinard V, Bornes S, Rochaix P, Tilkin AF, Peron JM, Bonnet J, et al. The expression of cd70 and cd80 by gene-modified tumor cells induces an antitumor response depending on the mhc status. Cancer Gene Therapy. 2000. 7: 1543-1556.

Eager R, Nemunaitis J. Gm-csf gene-transduced tumor vaccines. Molecular therapy: the Journal of the American Society of Gene Therapy. 2005. 12: 18-27.

Hollingsworth RE, Jansen K. Turning the corner on therapeutic cancer vaccines. NPJ Vaccines. 2019. 4: 7.

Jin MZ, Wang XP. Immunogenic cell death-based cancer vaccines. Frontiers in Immunology. 2021. 12: 697964.

Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-t immunotherapy for castration-resistant prostate cancer. The New England Journal of Medicine. 2010. 363: 411-422.

Kim HS. Development of genetically modified tumor cell containing costimulatory molecule. Biomed Sci Letters. 2019. 25: 398-406.

Kim HS. CD8-dependent tumor growth inhibition by tumor cells genetically modified with 4-1BBL. Biomed Sci Letters. 2021. 27: 329-333.

Kim SK, Cho SW. The evasion mechanisms of cancer immunity and drug intervention in the tumor microenvironment. Frontiers in Pharmacology. 2022. 13: 868695.

Kooreman NG, Kim Y, de Almeida PE, Termglinchan V, Diecke S, Shao NY, et al. Autologous ipsc-based vaccines elicit anti-tumor responses *in vivo*. Cell Stem Cell. 2018. 22: 501-513 e507.

Larocca C, Schlom J. Viral vector-based therapeutic cancer vaccines. Cancer Journal. 2011. 17: 359-371.

Lipson EJ, Sharfman WH, Chen S, McMiller TL, Pritchard TS, Salas JT, et al. Safety and immunologic correlates of melanoma gvax, a gm-csf secreting allogeneic melanoma cell vaccine administered in the adjuvant setting. Journal of Translational Medicine. 2015. 13: 214.

Lopes A, Vandermeulen G, Preat V. Cancer DNA vaccines: Current preclinical and clinical developments and future perspectives. Journal of Experimental & Clinical Cancer Research. 2019. CR 38: 146.

Munn DH, Bronte V. Immune suppressive mechanisms in the tumor microenvironment. Current Opinion in Immunology. 2016. 39: 1-6.

Rosenberg SA. Il-2: The first effective immunotherapy for human cancer. Journal of Immunology. 2014. 192: 5451-5458.

Sadeghi Najafabadi SA, Bolhassani A, Aghasadeghi MR. Tumor cell-based vaccine: An effective strategy for eradication of cancer cells. Immunotherapy. 2022. 14: 639-654.

Santos PM, Butterfield LH. Dendritic cell-based cancer vaccines. Journal of Immunology. 2018. 200: 443-449.

Sartoris S, Mazzocco M, Tinelli M, Martini M, Mosna F, Lisi V, et al. Efficacy assessment of interferon-alpha-engineered mesenchymal stromal cells in a mouse plasmacytoma model. Stem Cells and Development. 2011. 20: 709-719.

Schneble E, Clifton GT, Hale DF, Peoples GE. Peptide-based

cancer vaccine strategies and clinical results. Methods in Molecular Biology. 2016. 1403: 797-817.

Srivatsan S, Patel JM, Bozeman EN, Imasuen IE, He S, Daniels D, et al. Allogeneic tumor cell vaccines: The promise and limitations in clinical trials. Human Vaccines & Immunotherapeutics. 2014. 10: 52-63.

Tang T, Huang X, Zhang G, Hong Z, Bai X, Liang T. Advantages of targeting the tumor immune microenvironment over blocking immune checkpoint in cancer immunotherapy. Signal Transduction and Targeted Therapy. 2021. 6: 72.

Ullrich E, Bonmort M, Mignot G, Kroemer G, Zitvogel L. Tumor stress, cell death and the ensuing immune response. Cell Death and Differentiation. 2008. 15: 21-28.

Vinay DS, Ryan EP, Pawelec G, Talib WH, Stagg J, Elkord E, et al. Immune evasion in cancer: Mechanistic basis and therapeutic strategies. Seminars in Cancer Biology. 2015. 35 Suppl: S185-S198.

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