Biomedical Science Letters 2021, 27(4): 329~333
https://doi.org/10.15616/BSL.2021.27.4.329
eISSN : 2288-7415

Brief Communication

CD8-dependent Tumor Growth Inhibition by Tumor Cells Genetically Modified with 4-1BBL

Hong Sung Kim[†,*]

Department of Biomedical Laboratory Science, Korea Nazarene University, Cheonan 31172, Korea

We previously identified that tumor cells genetically modified with a 4-1BBL co-stimulatory molecule had anticancer effects in a CT26 mouse colorectal tumor model. To identify the distinction between immune cells in a mouse tumor model treated with tumor cells genetically modified with 4-1BBL or β-gal, we examined the immune cells in CT26-WT, CT26-βgal, and CT26-4-1BBL tumor bearing mice 21 days after tumor cell administration. The CD8+ T cells population in mice treated with tumor cells genetically modified with 4-1BBL was significantly increased on day 21 compared to that of tumor cells genetically modified with β-gal in the spleen and tumor tissue. The CD4+ T cell population was not different between the two mice groups. The Foxp3+CD25^{high} CD4 T cell population decreased on day 21 in tumor tissues, but the decrease was not significant. We also found that CD8 T cells had pivotal roles in inhibiting tumor growth by treating mice with ant-CD4 and CD8 antibodies. These results suggest that tumor cells genetically modified with 4-1BBL could inhibit tumor growth by affecting on CD8 T lymphocytes.

Key Words: CD8 T cells, Regulatory T cells, Tumor growth inhibition, 4-1BBL

4-1BBL activates T cells by binding 4-1BB on activated T cells as co-stimulatory molecule and derivess the expansion of T cell and the preservation of long-term memory function (Habib-Agahi et al., 2009; Wang et al., 2009; Vinay and Kwon, 2012; Mock et al., 2020; Trub et al., 2020). 4-1BBL is also related to potent tumor rejection effects in various mouse tumor model (Guinn et al., 2001; Stephan et al., 2007; Ragonnaud et al., 2016). Cytotoxic T cells were reported to be the major immune cell against tumor growth through 4-1BBL in tumor-bearing hosts (Yi et al., 2007; Kim et al., 2009; Lee and Kim, 2012; Sharma et al., 2014; Wang et al., 2015). Some research studies reported that 4-1BBL prevented cancer development via CD4+ T and natural killer cells (Madireddi et al., 2012; Barsoumian et al., 2019).

We previously constructed a lentiviral vector containing 4-1BBL and established tumor cells genetically modified with 4-1BBL and found that 4-1BBL expression on CT26 coloretal cancer cells was associated with tumor growth inhibition (Kim, 2019). In this study, we investigated that tumor growth inhibition by tumor cells genetically modified with 4-1BBL was related to CD8 T cell expansion and reductions in regulatory T cells. To identify this hypothesis, we analyzed the effect of tumor cells genetically modified with 4-1BBL on immune cells related to tumor growth inhibition 21 days after CT26 colorectal cancer cell inoculation and identified the effect of T lymphocytes on tumor growth inhibition using anti-CD4 and anti-CD8 antibody treatments.

Received: November 9, 2021 / Revised: November 22, 2021 / Accepted: November 22, 2021
[*]Professor.
[†]Corresponding author: Hong Sung Kim. Department of Biomedical Laboratory Science, Korea Nazarene University, Cheonan 31172, Korea.
Tel: +82-041-570-4165, Fax: 82-041-570-4258, e-mail: hskim@ kornu.ac.kr
©The Korean Society for Biomedical Laboratory Sciences. All rights reserved.
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Balb/c female mice (6 to 8-week-old) were obtained from OrientBio (Korea). The mice were nurtured under pathogen-free conditions and maintained by approved institutional animal care protocols. CT26 cells were obtained from ATCC (the American Type Culture Collection, Manassas, VA, USA) and were cultivated 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). CT26 mouse colorectal cancer cells with wild-type (CT26-WT), 4-1BBL (CT26-4-1BBL), and β-gal genes (CT26-βgal) (2×10^5) in 100 μL phosphate-buffered saline were seeded subcutaneously into the right flank of *Balb/c* mice. For CD4 and CD8 T cell depletion, CT26-4-1BBL tumor bearing mice were intraperitoneal injected with anti-CD4 and anti-CD8 antibodies (100 μg/mice) (eBioscience, San Diego, CA) six times (two times before tumor cell inoculation and four times after tumor cell inoculation). Tumor size was gauged in two dimensionsusing calipers and tumor area was calculated as follows: tumor area (mm^2) = length × width. The mice were sacrificed 21 days after tumor cell injection and the spleen and tumor tissues were aseptically collected. Each sample was homogenized and ran through a mesh to produce single-cell suspensions. After the cells were washed two times with PBS, RBC lysis buffer (140 nM ammonium chloride and 20 mM Tris-HCl) was treated by removing erythrocytes at 37℃ for 5 min and the cells were stained with the following mAbs: phycoerythrin (PE)-conjugated CD8 and CD25; allophycocyanin (APC)-conjugated anti-CD4; and fluorescein isothiocyanate (FITC)-conjugated FoxP3. All antibodies were acquired from eBiosciences (San Diego, CA, USA). Foxp3 Staining Buffer kit (eBioscience) was used for labeling splenocytes. Samples were examined using CELLquest™ software and a FACScalibur™ flow cytometer, and FACS data were analyzed by the Flowjo program and presented using Graphpad Prism6. The data are presented as the means ± SEM. Significance among the percentage of each cell were expressed using the two-tailed student's *t*-test. $P < 0.05$ were determined significant. To identify changes in systemic and tumor tissue immune cells in mice with tumor cells, we examined the lymphocytic populations (CD8, CD4, and Treg) in the spleen and tumor tissue. We collected splenocytes and tumor-infiltrating immune cells from tumor-bearing mice 21 days after tumor cell injection and implemented FACS analysis. In the spleen, the percentage of CD4+ and regulatory T cell populations in the CT26-WT,

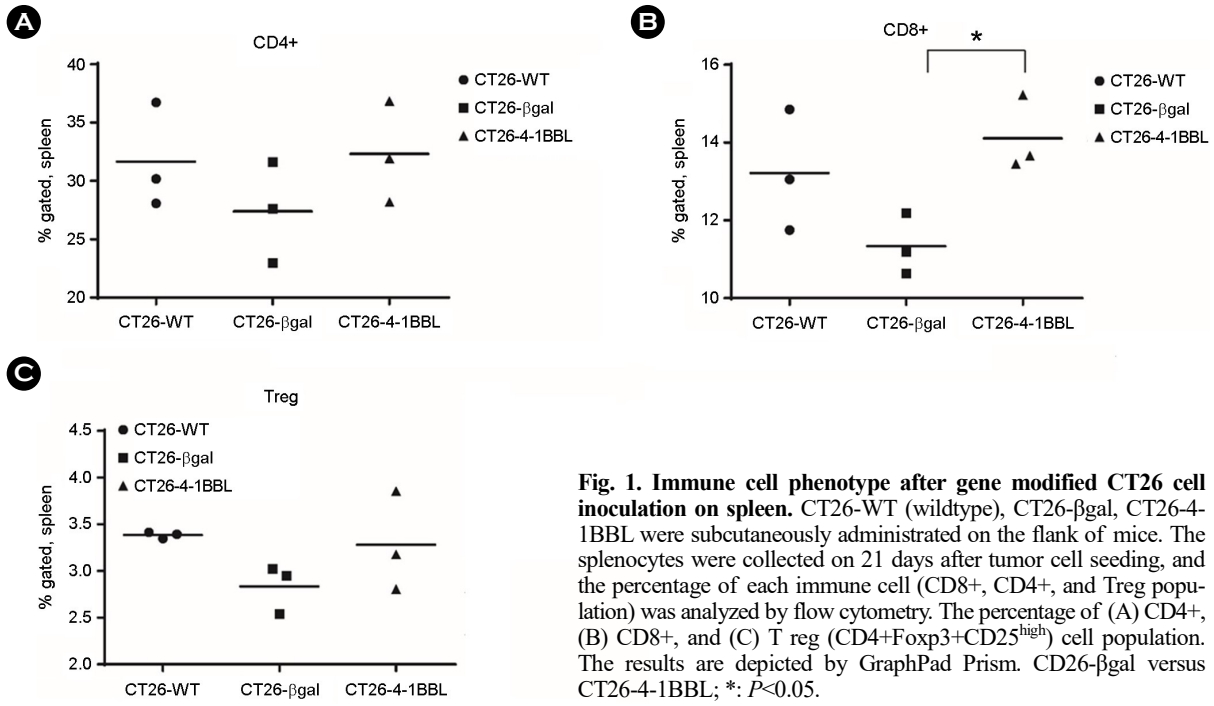

Fig. 1. Immune cell phenotype after gene modified CT26 cell inoculation on spleen. CT26-WT (wildtype), CT26-βgal, CT26-4-1BBL were subcutaneously administrated on the flank of mice. The splenocytes were collected on 21 days after tumor cell seeding, and the percentage of each immune cell (CD8+, CD4+, and Treg population) was analyzed by flow cytometry. The percentage of (A) CD4+, (B) CD8+, and (C) T reg (CD4+Foxp3+CD25^{high}) cell population. The results are depicted by GraphPad Prism. CD26-βgal versus CT26-4-1BBL; *: $P<0.05$.

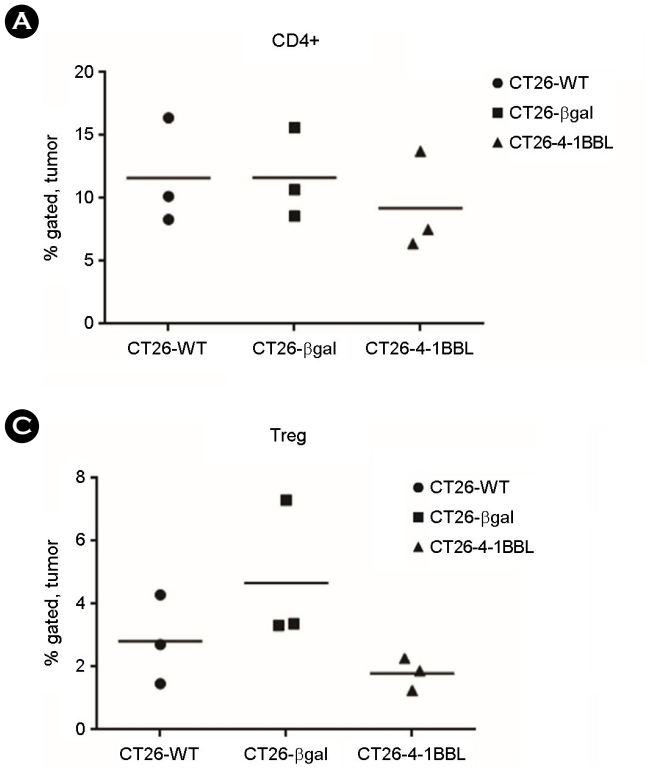

Fig. 2. Immune cell phenotype after gene modified CT26 cell inoculation on tumor tissue. Mice were treated by described Fig. 1. The tumor tissues were collected on 21 days after tumor cell seeding and the percentage of each immune cells was analyzed through flow cytometry. The percentage of (A) CD4+, (B) CD8+, and (C) T reg cell population. The results are depicted by GraphPad Prism. CD26-βgal versus CT26-4-1BBL; **: P<0.01.

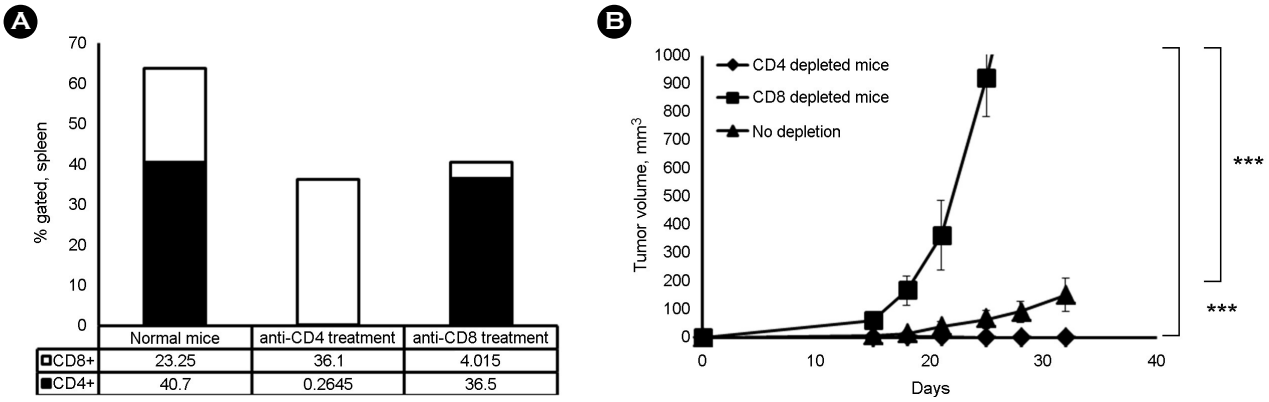

Fig. 3. Tumor growth pattern of CT26-4-1BBL bearing mice after antibody treatment. Before subcutaneous implantation of CT26-4-1BBl tumor cells (2×10^5 cell/100 μL PBS) on the flank of *Balb/c* mice, antibody was intraperitoneally treated two times every other day. Two days after tumor cell seeding, antibody was treated four times every week. The splenocytes were collected on 27 days after tumor cell seeding and the percentage of each immune cells was analyzed through flow cytometry. (A) The percentage of CD4+ and CD8+ T cell population. Tumor size was measured every third day. (B) The average tumor volume of each group. The results are defined as mean ± SEM. ***: P<0.0001.

CT26-βgal, and CT26-4-1BBL tumor-bearing mice were not significantly different (Figs. 1 A and 1C), but the percentage of CD8+ T cells in CT26-4-1BBL-bearing mice were significantly increased on day 21 compared to the CT26-βgal-bearing mice (Fig. 1B). In tumor tissues, the percentage of CD4+ and Treg cells were not significantly different among the CT26-WT, CT26-βgal, and CT26-4-1BBL tumor-bearing mice (Figs. 2 A and 2C), but CT26-4-1BBL mice showed a tendency toward decreased regulatory T cells compared to CT26-βgal mice (Fig. 2C). The percentage of CD8+ T cells in CT26-4-1BBL-bearing mice showed significant increases compare to the CT26-WT and

CD26-βgal-bearing mice (Fig. 2B). These data indicated that CT26-4-BBL induced CD8+ T cell population expansion in the spleen and tumor tissue. To identify the effect of CD8+ and CD4+ T cell population on tumor growth inhibition in CT26-4-1BBL-bearing mice, we treated them with anti-CD4 and anti-CD8 antibodies and identified the tumor growth patterns. On day 28, we identified immune cell depletion by antibody treatment (Fig. 3A). In the case of CD8 T cell-depleted mice, there was no tumor growth inhibition. The results indicated that CD8 T cells had a pivotal role in tumor growth inhibition in CT26-4-1BBL-bearing mice. In the case of CD4 T cell-depleted mice, there was significant tumor growth inhibition. Also, CD4 T cell-depleted mice showed better results compared to mice not treated with antibodies (Fig. 3B). These data represented that tumor growth inhibition in CT26-4-1BBL-tumor bearing mice was mainly dependent upon the existence of CD8+ T cells, especially in tumor tissues, and showed that CD4+ T cells could induce tumor growth in the CT26-4-1BBL system. In this study, we analyzed CD8, CD4, and Treg cells in mice with CT26-4-1BBL-bearing tumors. But there are many kinds of immune cells related to tumor growth inhibition including MDSC, NK cell, etc. In the future, we will investigate CD8 T cell subpopulations and other immune cells related to tumor growth inhibition.

ACKNOWLEDGEMENT

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), which is funded by the Ministry of Education, Science and Technology (2010005446), and by Korea Nazarene University Research Fund.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

Barsoumian HB, Batra L, Shrestha P, Bowen WS, Zhao H, Egilmez NK, Gomez-Gutierrez JG, Yolcu ES, Shirwan H. A novel form of 4-1BBL prevents cancer development via non-specific activation of CD4(+) T and natural killer cells. Cancer Research. 2019. 79: 783-794.

Guinn BA, Bertram EM, DeBenedette MA, Berinstein NL, Watts TH. 4-1BBL enhances anti-tumor response in the presence or absence of CD28 but CD28 is required for protective immunity against parental tumors. Cellular Immunology. 2001. 210: 56-65.

Habib-Agahi M, Jaberipour M, Searle PF. 4-1BBL costimulation retrieves CD28 expression in activated T cells. Cellular Immunology. 2009. 256: 39-46.

Kim HS, Kim-Schulze S, Kim DW, Kaufman HL. Host lympho-depletion enhances the therapeutic activity of an oncolytic vaccinia virus expressing 4-1BB ligand. Cancer Research. 2009. 69: 8516-8525.

Kim HS. Development of genetically modified tumor cell containing c0-stimulatory molecule. Biomedical Science Letters. 2019. 25: 398-406.

Lee NK, Kim HS. Oncolytic vaccinia virus expressing 4-1BBL inhibits tumor growth by increasing CD8+ T cells in B16F10 tumor model. Biomedical Science Letters. 2012. 18: 210-217.

Madireddi S, Schabowsky R-H, Srivastava AK, Sharma RK, Yolcu ES, Shirwan H. SA-4-1BBL costimulation inhibits conversion of conventiolnal CD4+ T cells into CD4+ FoxP3+ T regulatory cells by production of IFN-γ. PLoS One. 2012. 7: e42459.

Mock J, Stringhini M, Villa A, Weller M, Weiss T, Neri D. An engineered-4-1BBL fusion protein with "activity on demand". Proceedings of the National Academy of Sciences USA. 2020. 117: 31780-31788.

Ragonnaud E, Andersson AM, Pedersen AE, Laursen H, Holst PJ. An adenoviral cancer vaccine co-endoding a tumor associated antigen together with secreted 4-1BBL leads to delayed tumor progression. Vaccine. 2016. 34: 2147-2156.

Sharma RK, Yolcu ES, Shirwan H. SA-4-1BBL as a novel adjuvant for the development of therapeutic cancer vaccine. Expert Review of Vaccines. 2014. 13: 387-398.

Stephan MT, Ponomarev V, Brentjens RJ, Chang AH, Dobrenkov KV, Heller G, Sadelain M. T cell-encoded CD80 and 4-1BBL induce auto- and transcostimulation, resulting in potent tumor rejection. Nature Medicine. 2007. 13: 1440-1449.

Trub M, Uhlenbrock F, Claus C, Herzig P, Thelen M, Karanikas V, Bacac M, Amann M, Albrecht R, Ferrara-Koller C, Thommen D, Rothschield S, Savic Prince S, Mertz KD, Cathomas G, Rosenberg R, Heinzelmann-Schwarz V, Wiese M, Lardinosis D, Umana P, Klein C, Laubli H, Kashyap AS, Zippelius A.

Fibroblast activation protein-targeted-4-1BB ligand agonist amplifies effector functions of intratumoral T cells in human cancer. Journal for Immunotherpy of Cancer. 2020. 8: e000238.

Vinay DS, Kwon BS. Immunotherapy of cancer with 4-1BB. Molecular Cancer Therapeutics. 2012. 11: 1062-1070.

Wang C, Lin GH, McPherson AJ, Watts TH. Immune regulation by 4-1BB and 4-1BBL: complexities and challenges. Immunological Reviews. 2009. 229: 192-215.

Wang R, Freywald A, Chen Y, Xu J, Tan X, Xiang J. Transgenic 4-1BBL-engineered vaccine stimulates potent Gag-specific therapeutic and long-term immunity via increased priming CD44(+)CD62L(high)IL-7R(+) CTLs with up-and down-regulation of anti- and pro-apoptosis genes. Cellular & Molecular Immnology. 2015. 12: 456-465.

Yi KH, Nechushtan H, Bowers WJ, Walker GR, Zhang Y, Pham DG, Podack ER, Federoff HJ, Yolba KA, Rosenblatt JD. Adoptively transferred tumor-specific T cells stimulated *ex vivo* using herpes simplex virus amplicons encoding 4-1BBL persist in the host and show antitumor activity *in vivo*. Cancer Research. 2007. 67: 10027-10037.

https://doi.org/10.15616/BSL.2021.27.4.329

Cite this article as: Kim HS. CD8-dependent tumor growth inhibition by tumor cells genetically modified with 4-1BBL. Biomedical Science Letters. 2021. 27: 329-333.