공학박사 학위논문

Therapeutic Cancer Nanovaccine that Enhances T-cell Responses and Efficacy through Dual Interactions with Dendritic Cells and T Cells

수지상세포 및 T 세포와 동시에 상호작용하여 T 세포의 반응과 치료효능을 증진시키는 치료용 암 백신

2023 년 8 월

서울대학교 대학원

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Abstract

Therapeutic Cancer Nanovaccine that Enhances T-cell Responses and Efficacy through Dual Interactions with Dendritic Cells and T Cells

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Conventional cancer vaccines, which interact primarily with dendritic cells (DCs) to activate tumor-specific T cells, often fail to achieve sufficient therapeutic efficacy due to suboptimal activation of T cells. To address this problem, a therapeutic cancer nanovaccine that enhances T-cell responses by interacting with both DCs and T cells was developed in this study. This nanovaccine is composed of cancer cell membrane and lipids, forming a liposome-like nanoparticle (CCM-MPLA) that contains monophosphoryl lipid A (MPLA) as an adjuvant. To achieve direct interaction with tumor-specific T cells, anti-CD28 antibodies (aCD28) are conjugated with CCM-MPLA, resulting in CCM-MPLA-aCD28. This nanovaccine can improve the therapeutic efficacy of tumor-specific $CD8^+$ T cells in both presence and absence of DCs. CCM-MPLA-aCD28 induces more activation of tumor-

specific $CD8^+$ T cells and demonstrates higher anti-tumor efficacy in mice model comparing with conventional nanovaccines that interact with either DCs (CCM–MPLA) or T cells (CCM–aCD28). Additionally, no significant differences are observed in the level of T cell activation and therapeutic efficacy between CCM–MPLA and CCM–aCD28. This method may contribute to the development of effective personalized therapeutic cancer vaccines containing autologous cancer cell membranes.

Keyword : cancer immunotherapy, cancer vaccine, T cell, dendritic cell, cancer cell membrane

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Table of Contents

List of Figures

Chapter 1. Research backgrounds and purpose

1.1. Research backgrounds

1.1.1. Cancer immunotherapy

Cancer immunotherapy, strategies that helps immune systems to fight against cancer cells, has emerged as a promising therapy for cancer treatment over the years, [1-4] and there have been various approaches for cancer immunotherapy. First, immune check point inhibitors, such as inhibitors of programmed cell death 1 (PD-1), Programmed death-ligand 1, and cytotoxic T lymphocyte antigen 4 can be used for activation of cytotoxic T cells and increased antitumor efficacy.[5] Monoclonal antibodies, such as anti-CD20 and anti-HER2, can attach to the surface of cancer cells and induce their elimination. They can be used for cancer immunotherapy.[6] And, cell transfer therapies, which involve the transfer of T cells to tumor patients after ex vivo expansion and activation, have been proven to exhibit increased antitumor efficacy. These therapies, including conventional cytotoxic T-cell transfer and chimeric antigen receptor (CAR) T-cell transfer, have been widely used in cancer immunotherapy.[7] Therapies that modulate various cytokines that can act as immune modulators also have

been used for cancer immunotherapy.[8] The injection of inflammatory cytokines, such as interferon-alpha and interleukin-2, or the inhibition of anti-inflammatory cytokines, such as transforming growth factor-β, can activate the immune response against cancer cells. Last, cancer vaccines, which educate immune system to attack cancer cells, have been widely used for decades. These vaccines consequently induce antigen-specific T cells, which then eliminate cancer cells bearing the antigen.

1.1.2. Cancer vaccine

Cancer vaccine therapies have a distinct advantage compared to other cancer immunotherapies. These therapies grant antigen-specificity to cytotoxic T cells, enabling them to specifically target and attack cancer cells that express the antigen. As a result, they can reduce the side effects associated with T cells attacking normal cells. The antigens can take various forms, including proteins, peptides, and nucleic acids.[9] Moreover, cancer vaccines generally contain various kinds of molecules to enhance the immune response. Therefore, cancer vaccines can be engineered in various forms containing antigens inside. Various approaches have been used to develop therapeutic cancer vaccines.[10,11] Most of therapeutic cancer vaccines target antigen presenting cells (APCs). When these vaccines deliver the antigens to APCs, the APCs present the antigens in the form of major histocompatibility complex (MHC)–antigen complexes. These APCs interact with T cells via the interaction of the MHC-antigen complex and the T cell receptor (TCR), or through other T cell activation signals. One major hurdle in engineering cancer vaccines is the identification of antigens. Due to tumor heterogeneities and the presence of different types of cancer cells in each patient, finding specific antigens is challenging. Therefore, finding correct antigens or developing new methods to achieve antigen specificity is important to engineer cancer vaccine effectively.

1.1.3. DC targeting cancer vaccine

DC, one population of APCs, plays a central role for antigen-specific immune response.[12] DCs involve in both inflammatory immune response and anti-inflammatory immune response. After delivery of cancer vaccines to DCs, the DCs present MHC (class I or II)-antigen complex and express T-cell activation marker, CD80 and CD86. Afterward, activated tumor-specific cytotoxic T lymphocytes (CTLs) that migrate to tumor site and kill tumor cells.[13] Adjuvants, a molecules that enhance body's immune response, can be delivered together with tumor antigens to DCs to promote DC maturation, thereby enhancing their antigen-presenting ability and subsequently promoting tumor-specific T-cell activation. Most adjuvants are toll-like receptor (TLR) agonists, such as monophosphoryl lipid A (MPLA), CpG oligodeoxynucleotides, and polyinosinic: polycytidylic acid.[14] However, conventional therapeutic cancer vaccine strategies frequently have failed to demonstrate clinical benefits mainly due to suboptimal activation of tumor-specific $CD8^+$ T cells. It is difficult to specifically target only DCs and prevent diffusion into other sites of the body. Therefore, alternative approaches to activate antigen-specific T cells are needed to solve these problems.

1.1.4. Cancer vaccine interacting with T cells directly

To address the issue of suboptimal activation of T cells through DC targeting methods, a new approach for cancer vaccines has been previously developed. In T-cell activation mechanism, MHC-antigen complex and co-stimulation molecules of CD28, such as CD80 and CD86, ae essentially needed. Previous study induces tumor-specific T-cell activation through direct interaction between the vaccine and naïve $CD8^+$ T cells.[15] The cancer vaccine was fabricated by expressing CD80, a costimulatory signal, on the membrane of cancer cells and subsequently coating polymer nanoparticle cores with the cancer cell membrane. The cancer vaccine, which provides CD80 and presents tumor antigen epitopes on major histocompatibility complex (MHC) class I, was able to activate tumor-specific $CD8^+$ T cells by directly interacting with naïve T cells.[15] However, a considerable portion of the vaccine nanoparticles is readily engulfed by DCs, reducing the efficacy of T-cell activation. As a result, vaccines that activate tumor-specific T cells only by directly interacting with naïve T cells may not exhibit a difference in therapeutic efficacy compared with conventional vaccines that activate T cells via DCs.

1.2. Purpose of research

To handle this limitation, I developed a new method to make an effective tumor-specific cancer vaccine. This personalized therapeutic cancer vaccine can activate tumor-specific $CD8^+$ T cells through interactions with both DCs and T cells. I hypothesized that the vaccine that interacts with both DCs and T cells would exhibit higher efficacies in tumor-specific $CD8^+$ T cell activation and tumor inhibition. Thus, I used cancer cell membrane (CCM)-based nanoparticles to provide tumor antigens to DCs and T cells (Figure 1.1.). MPLA, an adjuvant approved by the U.S. Food and Drug Administration (FDA), was inserted into the CCM-based nanoparticle for DC activation.[16] Then, anti-CD28 antibodies (aCD28), which act as a T cell activation signal,[17] were conjugated to the vaccine to promote direct interaction with T cells.[18] Consequently, the nanovaccine (CCM–MPLA–aCD28) was designed to facilitate dual interactions with both DCs and T cells via different mechanisms. When the CCM–MPLA–aCD28 is intradermally injected to tumor-bearing mice, a portion of the CCM–MPLA–aCD28s would be absorbed by dermal dendritic cells (Figure 1.2.). Then, the DCs would migrate to the adjacent lymph nodes and express MHC class I-antigen complex, CD80, and CD86. The DCs activate naïve $CD8^+$ T cells in lymph nodes and induce tumor-specific $CD8^+$ T cells consequently. The remaining CCM–MPLA–aCD28 nanoparticles would diffuse into the adjacent lymph nodes and directly interact with naïve $CD8^+$

T cells through the MHC class I-antigen complex and aCD28, resulting in the activation of tumor-specific cytotoxic T lymphocytes (CTLs). CCM–MPLA–aCD28 can be used as a personalized cancer vaccine when it is prepared from autologous cancer cells, avoiding the need to identify certain tumor antigens for personalized vaccine preparation.

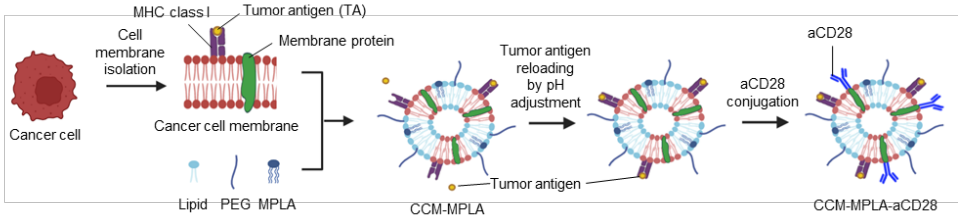

Figure 1.1. Schematic illustration depicting the stepwise preparation of CCM–MPLA–aCD28.

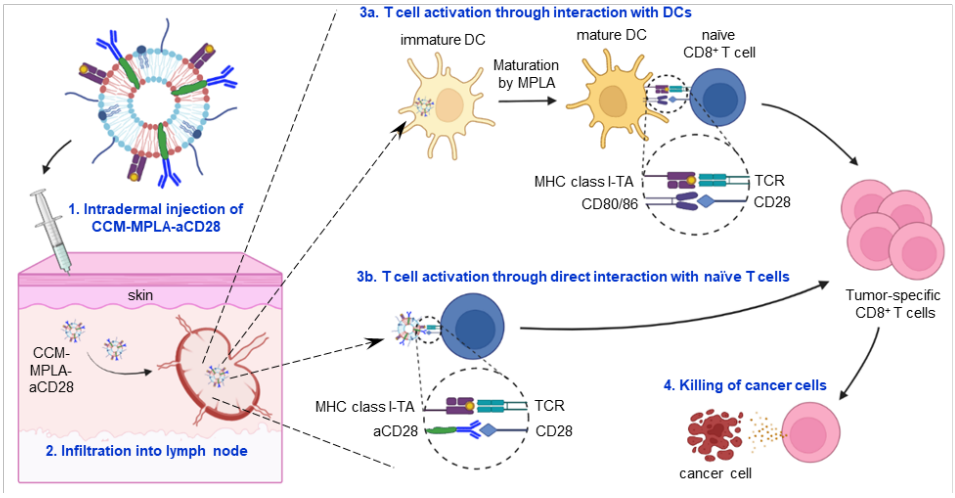

Figure 1.2. Schematic illustration describing the hypothesis of this study.

Chapter 2. Experimental section

2.1. Cell culture

2.1.1. Cell line culture

CT26, 4T1, and EL4 cells (all from American Type Culture Collection, Manassas, VA, USA) were cultured in Roswell Park Memorial Institute (RPMI) 1640 (Gibco, Grand Island, NY, USA) supplemented with fetal bovine serum (FBS, 10% (v/v); Gibco) and penicillin/streptomycin (PS, 1% (v/v); Gibco). E.G7-OVA cells, a variant of the EL4 cell line expressing the full-length OVA peptide, were a kind gift from Professor Junsang Doh, Seoul National University. The cells were cultured in RPMI 1640 supplemented with FBS [10% (v/v)], PS [1% (v/v)], HEPES (10 mM; Sigma Aldrich, St. Louis, MO, USA), sodium pyruvate (1.0 mM; Gibco), 2-mercaptoethanol (50 μM; Sigma Aldrich), and G418 solution (0.4 mg ml^{-1}; Roche, Basel, Switzerland). E.G7-OVA cells treated with recombinant mouse IFN-γ (0, 100, 500, and 1500 U ml^{-1}; BioLegend, San Diego, CA, USA) for 48 h were stained with APC-labeled mouse H-2Kb of the MHC class I bound to SIINFEKL (BioLegend) and analyzed using flow cytometry. Then, all the cancer cell lines (CT26, 4T1, EL4, and E.G7-OVA cells) were

incubated with IFN-γ (500 U ml^{-1}; BioLegend) for 48 h before harvesting for more number of antigen–MHC class I complexes on the membranes.

2.1.2. Primary cell isolation and culture

Bone marrow-derived dendritic cells (BMDCs) were isolated from the tibias and femurs of 6-week-old female C57BL/6, as described in a previous study[19], and were cultured in RPMI 1640 supplemented with FBS (10% (v/v)), PS (1% (v/v)), 2-mercaptoethanol (50 μM), and mouse granulocyte–macrophage colony-stimulating factor (20 ng ml^{-1}; BioLegend). Primary T cells were isolated from the lymph nodes and spleens of 6-week-old male OT-I mice using the MojoSort™ mouse naïve $CD8^+$ T cell isolation kit (BioLegend) according to the manufacturer's instructions. Then, primary T cells were cultured in RPMI 1640 supplemented with FBS (10% (v/v)), PS (1% (v/v)), GlutaMAX (1% (v/v); Gibco), HEPES (10 mM), sodium pyruvate (1.0 mM), and 2-mercaptoethanol (55 μM).

2.2. preparation of CCM-MPLA-aCD28

2.2.1. Isolation of cancer cell membrane

Plasma membranes were collected from cancer cells according to a previously described method with a few modifications.[20] Briefly, cancer cells were harvested and washed with PBS (pH 7.4). Then, the cell pellet was suspended in hypotonic cell lysis buffer containing Tris–HCl (20 mM, pH 7.5; Noble Biosciences, Gyeonggi, Korea), KCl (10 mM; Sigma Aldrich), $MgCl_2$ (2 mM; Sigma Aldrich), and EDTA-free SIGMAFAST™ Protease Inhibitor Cocktail (Sigma Aldrich, 1 tablet per 100 ml of solution) and disrupted by sonicating the samples at an amplitude of 10% for 25 times (sonication for 1 s and interval of 5 s on ice; Branson Sonifier, Branson Ultrasonic, CA, USA). To load the detached antigens onto MHC class I, sodium acetate (0.2 mM, pH 5.2; Sigma Aldrich) was added to the same volume of hypotonic cell lysis buffer and incubated for 30 min. After incubation, Tris–HCl (3.3% (v/v) 2 M, pH 11) was added to neutralize the solution. Then, the solution was centrifuged at 10,000 ×g and 4°C for 10 min to collect the supernatant. The supernatant was further centrifuged at 27,237 ×g and 4°C for 15 min. CCM was collected as a pellet. The pellet was resuspended in PBS (pH 7.4) for further experiments. The protein concentration of the isolated membrane was quantified using the Bradford reagent (Sigma Aldrich) according to the manufacturer's instructions.

2.2.2. Characterization of cancer cell membrane

To confirm the loading of antigens onto the MHC class I on the isolated membrane, OVA-unloaded and OVA-loaded E.G7-OVA cell membranes were stained with APC-labeled mouse H-2Kb of the MHC class I bound to SIINFEKL (BioLegend) and analyzed using flow cytometry. CT26 CCM was used for preparing CCM–MPLA–aCD28 for all animal experiments

2.2.3. Preparation of CCM-MPLA

CCM–MPLA–aCD28s were synthesized using a conventional thin-film hydration method.[21] 18:0 PC (1,2-distearoyl-sn-glycero-3-phosphocholine (A, 320 μg), Briefly, 18:0 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (80 μg), cholesterol (80 μg; Sigma Aldrich), and MPLA (20 μg) were mixed and evaporated to form a thin film. All lipids, except cholesterol, were purchased from Avanti Polar Lipids (Alabaster, AL, USA). The lipids were then hydrated with PBS containing CCM protein (500 μg) for 1 h and sonicated at an amplitude of 10% for 20 times (sonication for 1 s and intervals of 5 s on ice). The solution was then serially extruded through polycarbonate membrane filters with pore sizes of 400 μm, 200 nm, and 100 nm (Whatman, Maidstone, UK) using a mini-extruder (Avanti Polar Lipids). To load the detached antigens onto MHC class I, sodium acetate (0.2 mM,

pH 5.2; Sigma Aldrich) was added to the same volume of PBS (pH 7.4) containing CCM–MPLA and incubated for 30 min. After incubation, Tris–HCl (2.2% (v/v) 2 M, pH 11) was added to neutralize the solution. Then, the solution was centrifuged at 27,237 ×g and 4°C for 15 min to collect the pellet.

2.2.4. Preparation of CCM-MPLA-aCD28

The antibody conjugation method has been described previously.[22] Briefly, CCM–MPLA was resuspended in Traut's reagent (Thermo Fisher Scientific, Waltham, MA, USA) in PBS (pH 8) according to the manufacturer's instructions and washed twice with Tyrode's buffer comprising sodium chloride (134 mM), sodium bicarbonate (12 mM), potassium chloride (2.9 mM), disodium phosphate (0.34 mM), magnesium chloride hexahydrate (1 mM), and HEPES (10 mM). aCD28 (37.51, BioXcell, Lebanon, NH, USA) was incubated with Sulfo-SMCC (4.8 mg ml^{-1}; Thermo Fisher Scientific) for 2 h at 4°C. Sulfo-SMCC was removed using a centrifugal filter unit (10 kDa, Millipore, Burlington, MA, USA) before use. The CCM–MPLA and aCD28s were mixed, incubated for 2 h at room temperature in Tyrode's buffer, and washed twice before use.

2.2.5. Characterization of CCM-MPLA-aCD28

The morphology of CCM–MPLA–aCD28 was characterized using cryo-TEM (JEM-2100Plus, JEOL), which was operated at 200 kV after sample preparation with Vitrobot (Thermo Fisher Scientific). The hydrodynamic size distribution of CCM–MPLA–aCD28 in PBS or PBS mixed with the same volume of FBS was measured using dynamic light scattering (Zetasizer Nano ZS, Malvern Panalytical, Malvern, UK) for 120 h. The zeta potential of CCM–MPLA–aCD28s was measured using electrophoretic light scattering (Zetasized Nano ZS). ^{31}P-NMR analysis was conducted using AvanceIII-500 (Bruker, Billerica, MA, USA) to confirm the unilamellar structure of CCM–MPLA–aCD28s. $MnCl_2$ (0.2 mM) was used as an external shift reagent for CCM–MPLA–aCD28 (350 μg ml^{-1}) in D_2O.[23]

2.2.6. Analysis of CCM-MPLA-aCD28 composition

To confirm the loading of the $OVA_{257–264}$ peptides onto MHC class I on CCM (E.G7-OVA)–MPLA, CCM–MPLA was stained with APC-labeled mouse H-2Kb of the MHC class I bound to SIINFEKL and analyzed using flow cytometry. To ensure the conjugation of aCD28 with CCM–MPLA, FITC-labeled aCD28 (0, 10, 20, and 100 μg; BioLegend) was conjugated with CCM–MPLA (20 μg) and analyzed using flow cytometry. To verify the presence of the MHC class I–OVA complex and aCD28 on CCM–MPLA–aCD28, CCM–MPLA and CCM–MPLA–aCD28 conjugated with FITC-labeled aCD28 were stained with APC-labeled mouse H-2Kb of the

MHC class I bound to SIINFEKL and analyzed using flow cytometry. To ensure MPLA internalization, the Limulus amoebocyte lysate (Genscript Biotech, Singapore) test was conducted for CCM–MPLA–aCD28 and CCM–aCD28 according to the manufacturer's instructions.

2.3. In vitro assays

2.3.1. In vitro interaction of CCM-MPLA-aCD28s with DCs and T cells

To confirm the colocalization of DCs and T cells with CCM–MPLA–aCD28s, DCs or T cells stained with WGA (ThermoFisher Scientific, Waltham, MA, USA) were treated with CCM-MPLA-aCD28s labeled with DiI (Sigma Aldrich) for 18 h (DCs) or 2 h (T cells). Then, the cell nuclei were stained with a DAPI (Vector Laboratories, Burlingame, CA) and analyzed under a confocal laser microscope (Leica, Wetzlar, Germany).

2.3.2. In vitro viability test

To measure the cytotoxicity of CCM–MPLA–aCD28s toward DCs and T cells *in vitro*, the cells were treated with CCM–MPLA–aCD28 (0, 5, 50, and 500 μg) for 24 h. The relative number of viable DCs and T cells was measured using the Cell Counting Kit-8 assay (DoGenBio, Seoul, Korea) according to the manufacturer's instructions.

2.3.3. In vitro DC activation

To determine the effects of the vaccines on DCs *in vitro*, splenocytes were treated with PBS or CCM (50 μg), MPLA (50 μg), or CCM–MPLA (50 μg) for 24 h. The cells were then stained with FITC-labeled anti-mouse CD11c antibody (BioLegend), PE-labeled anti-mouse IA/IE antibody

(BioLegend), PE-labeled anti-mouse CD80 antibody (BioLegend), and APC-labeled anti-mouse CD86 antibody (BioLegend). Thereafter, BMDCs were treated with CCM (E.G7-OVA)–MPLA–aCD28s (0, 10, 25, 50, and 100 μg) for 24 h and stained with APC-labeled mouse H-2Kb of the MHC class I bound to SIINFEKL (BioLegend). The cells were analyzed using flow cytometry.

2.3.4. In vitro T cell proliferation and activation

To determine the ability of CCM–MPLA–aCD28 to directly activate $CD8^+$ T cells, $CD8^+$ T cells from OT-I transgenic mice were isolated via magnetic separation using the CD8 isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany) and labeled with CTV (1 μM; ThermoFisher Scientific, Waltham, MA, USA) for 20 min at 37°C. The cells were seeded at 2×10^5 cells/well, followed by treatment with PBS or CCM derivatives (10 μg ml^{-1}). In some experiments, OT-I $CD8^+$ T cells (1×10^5 cells/well) were cocultured with BMDCs (1×10^4) and treated with CCM, CCM–aCD28, CCM–MPLA, irCCM–MPLA–aCD28, and CCM–MPLA–aCD28 (all at a concentration of 50 μg ml^{-1}) for 24 h. Otherwise, OT-I $CD8^+$ T cells (2×10^5 cells/well) were cocultured with wild-type splenocytes (2×10^6) and treated with CCM–MPLA–aCD28 (10 μg ml^{-1}) to determine the effect of CCM–MPLA–aCD28 in a diverse immune cell environment. The proliferative ability of the cells was analyzed on day 3 using flow cytometry.

The proliferation index was calculated as the total number of divisions/cells that went into division.

2.4. In vivo antitumor efficacy of CCM-MPLA-aCD28

2.4.1. In vivo DC activation

To determine the effects of the vaccine on DCs *in vivo*, CT26 cancer cells (5×10^5 cells) were subcutaneously injected into BALB/c mice. PBS, CCM–aCD28, CCM–MPLA, irrelevant cancer cell membrane (irCCM)–MPLA–aCD28, and CCM–MPLA–aCD28 (100 μg of vaccine per mouse) were intradermally injected into mice on days 5, 10, and 15 after tumor injection. Draining lymph nodes and spleens were harvested 16 days after tumor injection, mechanically disrupted using a homogenizer, and incubated with ACK Lysing Buffer (Gibco) for 30 s. After washing two times, the single-cell suspensions were passed through a 40-μm nylon mesh and stained with FITC-labeled anti-mouse CD11c antibody (BioLegend), PE-labeled anti-mouse CD80 antibody (BioLegend), and APC-labeled anti-mouse CD86 antibody (BioLegend). The cells were then analyzed using flow cytometry.

2.4.2. Biodistribution of CCM–MPLA–aCD28

To observe the distribution of CCM–MPLA–aCD28 after intradermal injection, CT26 cancer cells (5×10^5 cells) were subcutaneously injected into BALB/c mice. After 10 days of injection, 1,1′-dioctadecyl-3,3,3′,3′-

tetramethylindodicarbocyanine, 4-chlorobenzenesulfonate (DiD, 100 μg; Invitrogen, CA, USA)-labeled CCM–MPLA–aCD28 was intradermally injected. After 24 h, fluorescence images of the whole body or five major organs (heart, lungs, liver, kidneys, and spleen) and lymph nodes were obtained using the IVIS Spectrum computed tomography (PerkinElmer, USA).

2.4.3. In vivo interaction of CCM–MPLA–aCD28 with DCs and T cells

To confirm the colocalization of CCM–MPLA–aCD28 with DCs and T cells in the lymph node, the mice that were intradermally injected with DiD-labeled CCM–MPLA–aCD28 (100 μg) were euthanized after 24 h. Lymph nodes were harvested and fixed with 4% PFA, immersed in 30% sucrose, and embedded in an OCT compound (Scigen Scientific, Gardena, CA, USA). The tissues were then sectioned using a cryostat microtome (Leica, Wetzlar, Germany), followed by IHC staining using antibodies against CD11c (a DC marker) or CD3 (a T cell marker). The tissues were stained with DAPI and analyzed under confocal laser scanning and confocal laser microscopes (Leica).

2.4.4. In vivo toxicity test

To assess the toxicity of CCM–MPLA–aCD28 *in vivo*, CT26 cancer cells (5×10^5 cells) were subcutaneously injected into BALB/c mice. Mice were intradermally injected with PBS or CCM–MPLA–aCD28 (100 μg) at 5,

10, and 15 days after tumor injection. On day 20, five major organs were harvested, fixed with 4% PFA, immersed in 30% sucrose, and embedded in an OCT compound (Scigen Scientific, Gardena, CA, USA). Then, the organs were sectioned using a cryostat microtome (Leica) and stained with H&E. Images of the sectioned tissues were obtained using an optical microscope (Olympus, Tokyo, Japan). To assess hepatotoxicity and renal toxicity, mice were injected with PBS or CCM–MPLA–aCD28 (100 μg) on days 0, 5, and 10. Whole blood samples were collected on days −1, 1, 6, 11, and 18. The serum was isolated from whole blood samples. AST, ALT, BUN, and creatinine were determined using the DRI-CHEM 3500S chemistry analyzer (Fujifilm, Japan).

2.4.5. In vivo tumor growth

To confirm the efficacy of the vaccines *in vivo*, CT26 cancer cells (5×10^5 cells) were subcutaneously inoculated into BALB/c mice. The mice were intradermally injected with PBS or CCM–aCD28, CCM–MPLA, irCCM–MPLA–aCD28, or CCM–MPLA–aCD28 (100 μg) 5, 10, or 15 days after tumor injection. Tumor volumes were measured every 2–3 days and calculated using the following formula: [0.5 × (longest diameter) × (shortest diameter)^2]. On day 20, the tumors of the mice were harvested and weighed. Then, the tumors were fixed with 4% PFA, immersed in 30% sucrose, and embedded in an OCT compound. Tissues were sectioned using a cryostat

microtome (Leica) and stained with TUNEL (DeadEndTM TUNEL System, Promega, Madison, WI, USA) according to the manufacturer's instructions.

2.4.6. TIL analysis

TIL analysis was conducted according to a previous study.[24] Briefly, samples were digested with RPMI 1640 medium containing FBS (10%, Gibco), penicillin/streptomycin (1%, Gibco) (RF10), collagenase IV (0.5 mg ml^{-1}, Gibco), dispase (2 mg ml^{-1}, Gibco), and DNase I (30 μg ml^{-1}; Bio Basic) for 30 min at 37°C, with gentle agitation using the gentleMACS^{TM} Dissociator (Miltenyi Biotec). After disruption, the single-cell suspensions were passed through a 70-μm nylon mesh and then resuspended in Percoll density gradient media (GE Healthcare, Chicago, IL, USA). The cells in the interphase between Percoll and media were collected, washed with RPMI 1640 medium containing FBS (2% (v/v)), and analyzed using flow cytometry.

2.4.7. Animal study approval

All animal experiments performed in the present study were approved by the Institutional Animal Care and Use Committee of Seoul National University (SNU-220331-3 and SNU-220127-3-1). OT-I transgenic mice (C57BL/6-Tg(TcraTcrb)1100Mjb/Crl) were a kind gift from Professor Chang-Yuil Kang, Seoul National University. All experiments were performed on 6-week-old female mice as previously described. The BALB/c

and C57BL/6 mice were purchased from JA Bio (Gyeonggi, Republic of Korea).

2.4.8. Statistical analyses

Statistical comparisons were performed using the unpaired t-test for two-group comparisons, one-way analysis of variance (ANOVA) with Tukey's significant difference post-hoc test was used for comparisons of more than three groups, and two-way ANOVA with Bonferroni's multiple comparison tests were used for comparisons of two independent variables. All statistical analyses were performed using Prism 8 (GraphPad software). Differences were considered statistically significant when the p-value < 0.05.

Chapter 3. CCM-MPLA-aCD28 for cancer treatment

3.1. Characterization of CCM-MPLA-aCD28

CCM–MPLA–aCD28 was fabricated by fusing CCM and liposomes and subsequently conjugating aCD28. First, CCM, which contains tumor antigens loaded on MHC class I, was isolated from cancer cells using the hypotonic lysis method.[25] Lipids and MPLA were then mixed and evaporated to form a thin film. The thin film was hydrated with CCM to hybridize it with the liposomes, followed by serial extrusion, resulting in the formation of CCM–MPLA. Thereafter, aCD28 was conjugated to CCM-MPLA using the thiol–maleimide reaction to generate CCM–MPLA–aCD28.

Cryogenic-transmission electron microscopic (Cryo-TEM) images revealed that CCM–MPLA–aCD28 has a spherical shape and unilamellar lipid bilayer structure (Figure 3.1.A.). Dynamic light scattering (DLS) and electrophoretic light scattering (ELS) revealed that CCM–MPLA–aCD28 has a hydrodynamic diameter of 124.3 ± 8.1 nm and a zeta potential of −5.5 ± 0.6 mV, respectively (Figure 3.1.B.). It was reported that particles with diameters <200 nm can easily diffuse into the lymph nodes.[26] Therefore, the size of CCM–MPLA–aCD28 is permissive for interaction with T cells in the lymph nodes after intradermal injection of CCM-MPLA-aCD28. CCM–

MPLA–aCD28 in a serum-containing buffered solution did not exhibit significant changes in size (Figure 3.1.C.), suggesting that aggregation did not occur after *in vivo* injection. To confirm the unilamellar structure of CCM–MPLA–aCD28, ^{31}P-NMR analysis was performed (Figure 3.1.D.). A previous study reported that multilamellar vesicles show a very broad ^{31}P-NMR spectrum owing to restricted anisotropic motion, whereas unilamellar vesicles are characterized by a narrow line spectrum.[27,28] The ^{31}P-NMR data for CCM–MPLA–aCD28 revealed a narrow peak, indicating a unilamellar structure. Furthermore, Mn^{2+} was used as an external NMR shift reagent. Because the shift reagent only interacts with the phospholipids located in the outermost monolayer, the elimination of the peak in the ^{31}P-NMR spectra after Mn^{2+} addition indicates that CCM–MPLA–aCD28 has a unilamellar structure.

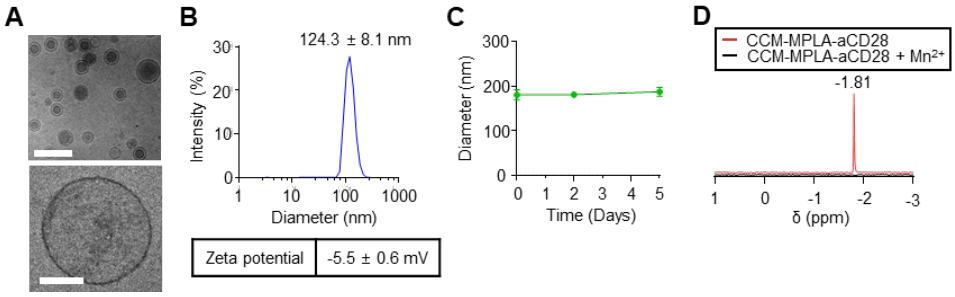

Figure 3.1. Characterization of CCM-MPLA-aCD28. (A) Cryo-transmission electron microscopic images showing the sphere-shaped structures of CCM–MPLA–aCD28. Scale bars: 500 nm (upper), 50 nm (lower). (B) Hydrodynamic size distribution, average size, and surface zeta potential of CCM–MPLA–aCD28, as determined using dynamic light scattering DLS/ELS. (C) Colloidal stability of CCM–MPLA–aCD28 at 4°C in 50% (v/v) serum, as analyzed using DLS; (n = 3). *$p < 0.05$. (D) ^{31}P-NMR analysis showing that CCM–MPLA–aCD28 has a unilamellar liposomal structure. $MnCl_2$ was used as an external shift reagent. (B, C) Data are presented as mean ± SD.

3.2. Composition of CCM-MPLA-aCD28

Next, I evaluated the presence of tumor antigens on the MHC class I of the CCM in E.G7-OVA cells using an antibody that detects the complex between the epitope of ovalbumin (OVA) and MHC class I. Because peptides that are loaded on MHC class I are readily dissociated during cell membrane isolation and liposome fabrication, I reloaded the tumor antigens on MHC class I by adjusting the pH during CCM–MPLA preparation as a previous study did.[22] The antigenic peptide reloading process allowed efficient reloading of the peptides onto the MHC class I of the CCM nanoparticles (Figure 3.2.A.) and CCM–MPLA nanoparticles (Figure 3.2.B.). Flow cytometric analysis was conducted to determine the optimal mass ratio of CCM proteins to aCD28 during the conjugation of aCD28 on CCM-MPLA. An equal mass ratio (1:1) of CCM protein to aCD28 was used during CCM–MPLA–aCD28 fabrication because this ratio was the minimum ratio that resulted in aCD28 conjugation to almost 100% of CCM–MPLA (Figure 3.2.C.). I demonstrated that 46.6 % of CCM–MPLA–aCD28s contained both the tumor antigen–MHC class I complex and aCD28 on their surfaces (Figure 3.2.D.). Next, to confirm whether MPLA, a detoxified form of the endotoxin lipopolysaccharide, was incorporated into CCM–MPLA–aCD28, the Limulus amebocyte lysate (LAL) endotoxin assay was performed. CCM–MPLA–aCD28 exhibited higher endotoxin levels than CCM–aCD28 (Figure 3.2.E.), indicating that MPLA was

appropriately incorporated into CCM–MPLA–aCD28.

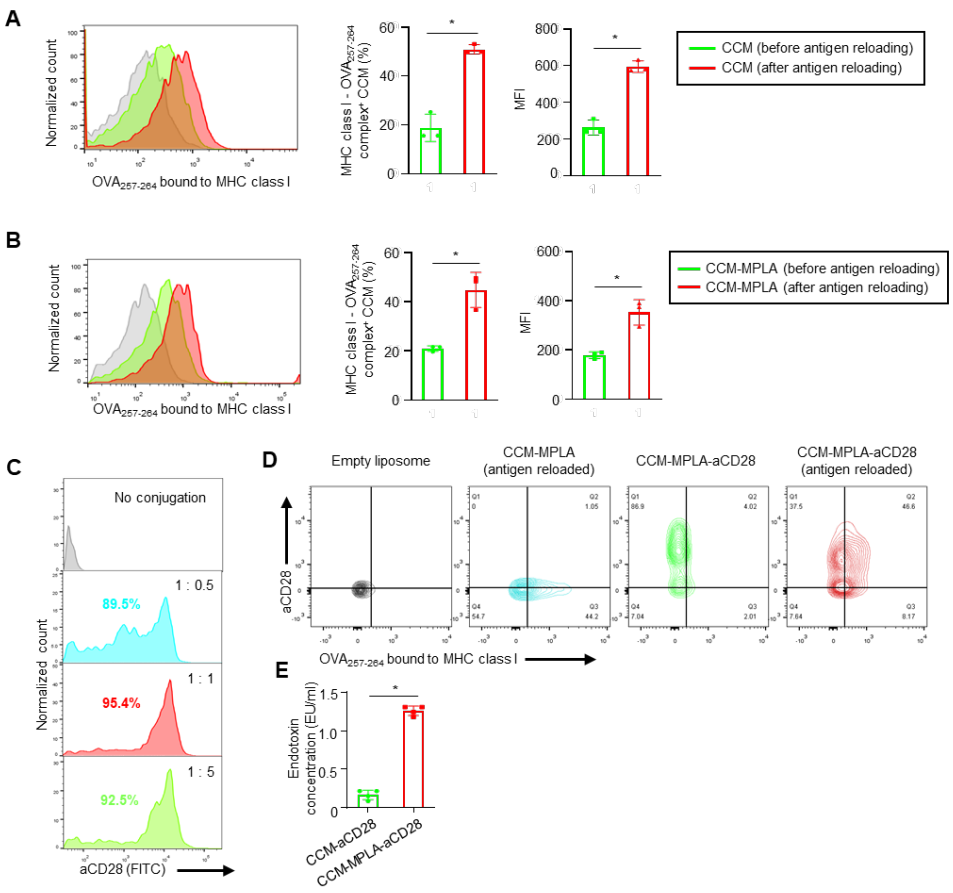

Figure 3.2. Composition of CCM-MPLA-aCD28. The percentages of CCMs or CCM–MPLAs that present the tumor antigen peptide ($OVA_{257-264}$) on MHC class I of (A) isolated CCM (E.G7-OVA) and (B) CCM–MPLA before and after antigen reloading via pH adjustment, as evaluated using antibodies against the MHC class I–$OVA_{257-264}$ complex and flow cytometry (n = 3). The gray histograms indicate isotype antibody controls. MFI means mean fluorescence intensity. *$p < 0.05$. (C) Percentage of CCM–MPLA on which aCD28 is conjugated after the aCD28 conjugation process at various ratios. The ratio indicates the mass ratio of the proteins in CCM to aCD28. (D) Colocalization of tumor antigens on the MHC class I and aCD28 on

CCM–MPLA–aCD28, as evaluated using flow cytometry. (E) LAL endotoxin assay showing that MPLA is present in CCM–MPLA–aCD28 but not in CCM–aCD28 ($n = 4$). *$p < 0.05$. (A, B, and E) Data are presented as mean ± SD.

3.3. Interaction of CCM-MPLA-aCD28 with DCs

I hypothesized that CCM–MPLA–aCD28 can activate tumor-specific T cells via two pathways: (1) interacting with DCs and (2) directly interacting with naïve T cells (Figure 1b). First, we investigated whether CCM–MPLA–aCD28s can activate antigen-specific T cells by interacting with DCs *in vitro* and *in vivo*. To verify whether CCM–MPLA–aCD28 was engulfed by DCs, wheat germ agglutinin (WGA)-stained DCs were treated with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine (DiI)-labeled CCM–MPLA–aCD28 for 18 h and stained with 4′,6-diamidino-2-phenylindole (DAPI). Confocal microscopic analysis revealed that CCM–MPLA–aCD28s were effectively engulfed by DCs (Figure 3.3.A.). CCM–MPLA–aCD28s exhibited no cytotoxicity toward DCs (Figure 3.3.B.). I treated DCs with various agents and evaluated the expression of the DC activation markers such as MHC class II, CD86, and CD80. CCM–MPLA, which not only presents antigens to DCs but also stimulates them via the TLR4 signaling pathway by MPLA, increased the expression of the DC activation markers (Figure 3.3.C.). CCM–MPLA–aCD28 was prepared using the CCM derived from E.G7-OVA cells. As the treatment dose of CCM–MPLA–aCD28 increased to 50 μg ml^{-1}, the expression level of the MHC class I-OVA complex on the DCs increased in a dose-dependent manner until the concentration of CCM-MPLA-aCD28 reaches 50 μg ml^{-1} (Figure 3.3.D.), indicating that the uptake of CCM–MPLA–aCD28 by DCs

leads to the presentation of CCM-derived antigens on the MHC class I of DCs. To determine whether CCM–MPLA–aCD28 can activate DCs *in vivo*, we intradermally administered CCM–MPLA–aCD28 into mice and analyzed DCs in the lymph nodes and spleens. CCM–MPLA- and CCM–MPLA–aCD28-treated mice exhibited the highest levels of CD80 and CD86 (DC activation markers) (Figure 3.3.E.), indicating that both CCM and MPLA are needed for the *in vivo* DC activation. Taken together, the results indicate that CCM–MPLA–aCD28 can present antigens to DCs and induce the activation of DCs *in vitro* and *in vivo*.

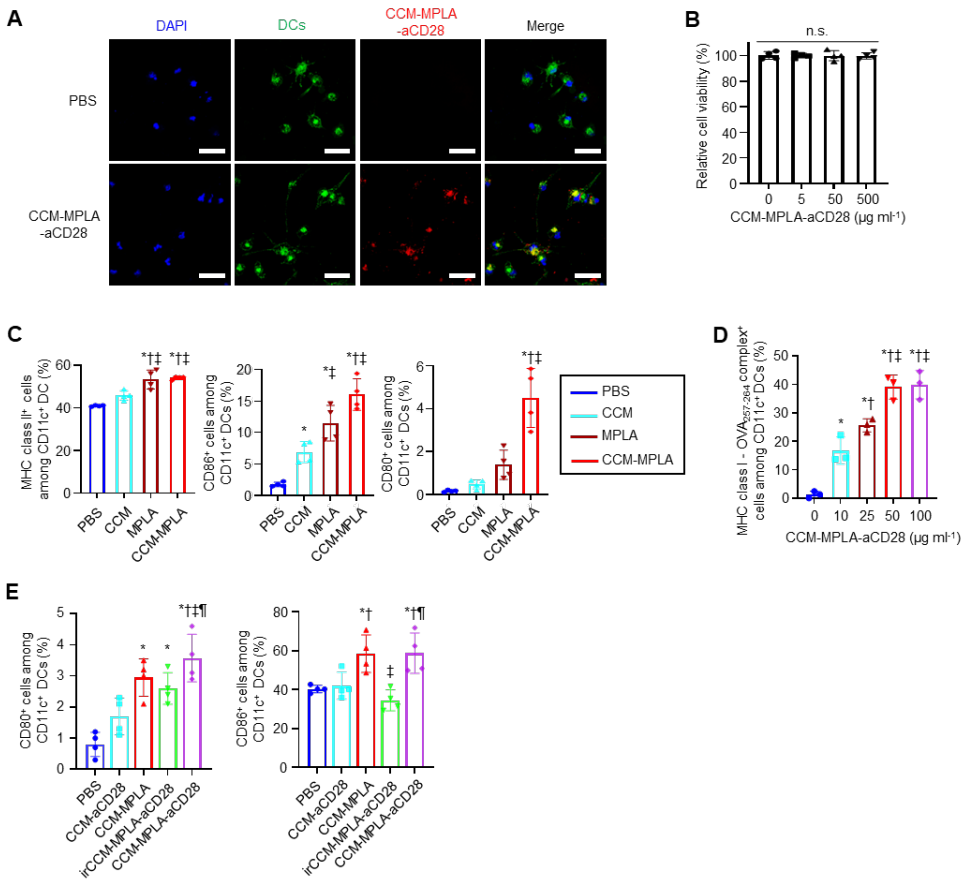

Figure 3.3. Interaction of CCM-MPLA-aCD28 with DCs. CCM was derived from EG.7-OVA cells. The irrelevant cancer cell membrane (irCCM) was derived from EL4 cells. (A) Fluorescence microscopic images show that CCM–MPLA–aCD28 is engulfed by immature DCs *in vitro*. Scale bar: 100 μm. (B) Cell viability of DCs treated with various concentrations of CCM–MPLA–aCD28 for 24 h, as evaluated using the Cell Counting Kit-8 assay (n = 4). n.s.: not significant. (C) Flow cytometric analysis showing the expression of DC activation makers (e.g., MHC class II, CD86, and CD80) *in vitro* (n = 4). *p < 0.05 versus PBS, †p < 0.05 versus lipopolysaccharide, ‡p < 0.05 versus CCM, and ¶p < 0.05 vs MPLA.

(D) Treatment of DCs with CCM–MPLA–aCD28 can induce CCM-derived antigen presentation on the MHC class I of DCs in a dose-dependent manner (n = 3). *$p < 0.05$ versus PBS, ‡$p < 0.05$ versus positive control, †$p < 0.05$ versus 10 μg ml^{-1}, and ¶$p < 0.05$ versus 25 μg ml^{-1}. (E) CCM–MPLA–aCD28-treated mice exhibited higher expression of the activation markers CD80 and CD86 in the DCs of the lymph nodes and spleens (n = 4). * $p < 0.05$ versus PBS, †$p < 0.05$ versus CCM–aCD28, ‡$p < 0.05$ versus CCM–MPLA, and ¶$p < 0.05$ versus irCCM–MPLA–aCD28. (B-E) Data are presented as mean ± SD. n.s.: not significant

3.4. Interaction of CCM-MPLA-aCD28 with T cells

Next, I elucidated whether CCM–MPLA–aCD28 can activate $CD8^+$ T cells by directly interacting with $CD8^+$ T cells. To demonstrate that CCM–MPLA–aCD28s can interact with T cell surfaces, WGA-labeled T cells were treated with DiI-labeled CCM–MPLA–aCD28s. Confocal microscopy revealed the direct interaction between CCM–MPLA–aCD28 and the $CD8^+$ T cell surface (Figure 3.4.A.). CCM–MPLA–aCD28 did not exhibit any notable cytotoxicity toward T cells *in vitro* (Figure 3.4.B.). Next, to determine whether CCM–MPLA–aCD28 can activate antigen-specific $CD8^+$ T cells, we treated CellTracker Violet (CTV)-labeled naïve $CD8^+$ T cells, isolated from OT-I mice that exhibit $CD8^+$ T cell receptors (TCR) specific to the $OVA_{257–264}$ epitope[29], with CCM–MPLA–aCD28. The CCM was derived from E.G7-OVA cells. CCM–aCD28- and CCM–MPLA–aCD28-treated T cells exhibited the highest percentage of interleukin (IL)-2^+ CTV^{low} $CD8^+$ T cells among all the groups (Figure 3.4.C.), indicating that these nanoparticles induced the highest degree of activation and proliferation of antigen-specific $CD8^+$ T cells. Together, these data indicate that both antigen–MHC class I–TCR and aCD28–CD28 interactions are necessary for activating and proliferating antigen-specific $CD8^+$ T cells. CCM–MPLA–aCD28 treatment increased the proliferation of antigen-specific $CD8^+$ T cells in a dose-dependent manner (Figure 4.4.D.). Flow cytometry was used to determine the activation of tumor antigen-specific

$CD8^+$ T cells treated with different groups of cancer vaccines. CCM–MPLA–aCD28 highly upregulated the T cell activation markers CD44 and CD69 (Figure 3.4.E. and 3.4.F.). This indicates that both cognate tumor antigens and aCD28 on cancer vaccines are required for effectively activating tumor-specific $CD8^+$ T cells by directly interacting with $CD8^+$ T cells. Taken together, these data suggest that CCM–MPLA–aCD28 directly interacts with and activates antigen-specific $CD8^+$ T cells.

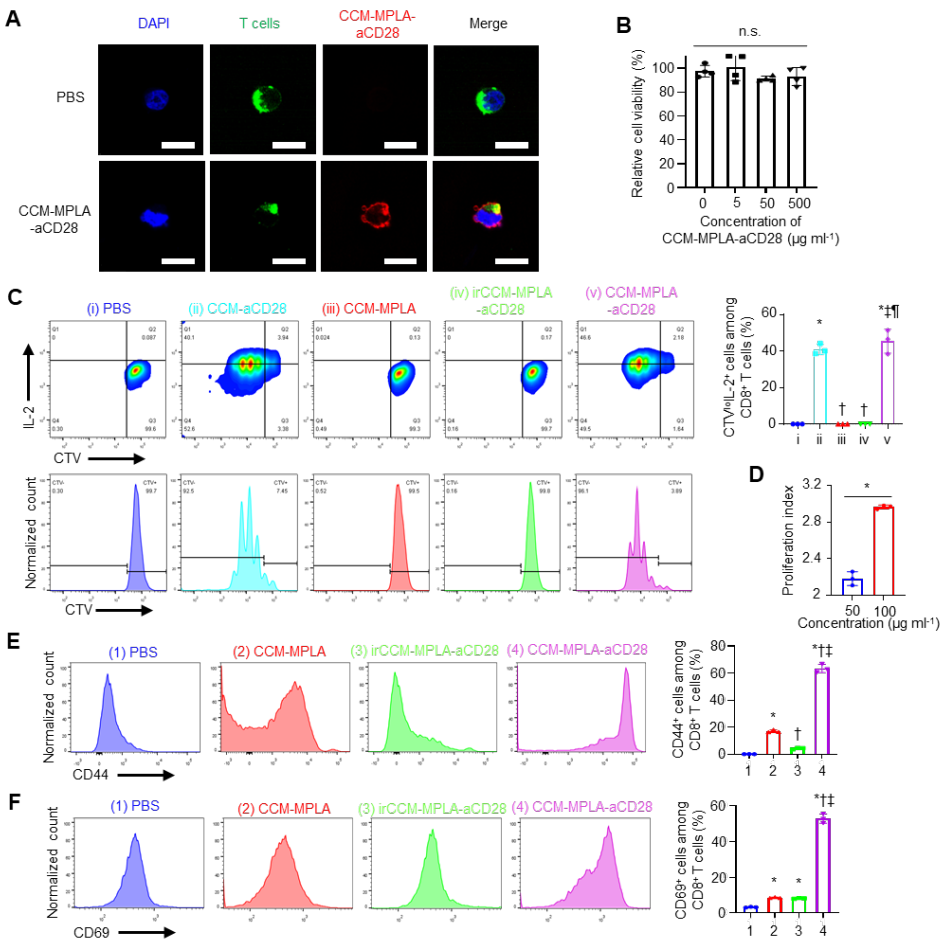

Figure 3.4. Interaction of CCM-MPLA-aCD28 with T cells. CCM was derived from EG7-OVA cells. irCCM was derived from EL4 cells. T cells were isolated from OT-I mice. (A) Fluorescence microscopic images show that CCM–MPLA–aCD28 interacts with the naïve T cell surface after 30-min treatment. Scale bar: 20 μm. (B) CCM–MPLA–aCD28 does not exhibit cytotoxicity toward T cells for 24 h (n = 4). Flow cytometry showing OT-I T cell proliferation after treatment (C) with different agents (D) at different doses for 3 days (n = 3). (C) *$p < 0.05$ versus (i), † $p < 0.05$ versus (ii), ‡$p < 0.05$ versus (iii), and ¶$p < 0.05$ versus (iv). (D) *$p < 0.05$ versus 50 μg ml^{-1}

of (ii), † $p < 0.05$ versus 100 μg ml^{-1} of (ii), and ‡$p < 0.05$ versus 50 μg ml^{-1} of (v). (E, F) Expression of the T cell activation markers (E) CD44 and (F) CD69 on OT-I T cells after treatment with various agents for 4 days (n = 3), indicating that both cognate tumor antigens and aCD28 on cancer vaccines are required for the effective activation of tumor-specific $CD8^+$ T cells via direct interaction with $CD8^+$ T cells. *$p < 0.05$ versus (1), † $p < 0.05$ versus (2), and ‡$p < 0.05$ versus (3). Data are presented as mean ± SD. (B-F) Data are presented as mean ± SD.

3.5. Interaction of CCM-MPLA-aCD28 with DCs and T cells

To determine whether CCM–MPLA–aCD28-treated DCs can activate T cells, DCs treated with various agents were cocultured with CTV-labeled naïve $CD8^+$ T cells harvested from OT-I mice. DCs treated with either CCM–MPLA or CCM–MPLA–aCD28 exhibited higher proliferation of T cells and expressed higher levels of CD44 (a T cell activation marker) on T cells than the other groups (Figure 3.5.A.). To imitate the *in vivo* environment in which DCs and T cells are present, DCs derived from wild-type C57BL/6 mice were treated with various agents and subsequently cocultured with OT-I T cells for 3 days. CCM–MPLA–aCD28 treatment resulted in the highest levels of T cell proliferation and activation (Figure 3.5.B.). The CCM–aCD28 and CCM–MPLA groups, which activate T cells by interacting with either T cells or DCs, exhibited lower levels of T cell activation and proliferation than CCM–MPLA–aCD28. Importantly, these data indicate that CCM–MPLA–aCD28 that interact with both DCs and T cells induces more potent T cell immunity than vaccines that interact with either DCs or T cells. Taken together, the data in Figure 3.3, 3.4, and 3.5 suggest that CCM–MPLA–aCD28s can efficiently activate tumor-specific T cells by interacting with both DCs (Figure 3.3), naïve T cells (Figure 3.4), and both DCs and T cells (Figure 3.5.).

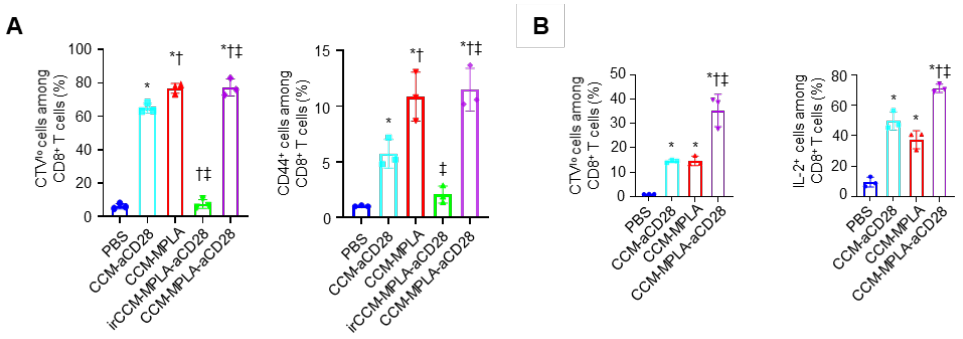

Figure 3.5. Interaction of CCM-MPLA-aCD28 with DCs and T cells. CCM was derived from EG7-OVA cells. irCCM was derived from EL4 cells. T cells were isolated from OT-I mice. (A) The percentage of T cells proliferating(CTV^{low}) and expressing CD44, a T cell activation marker, in OT-I mice-derived $CD8^+$ T cells after coculturing for 3 days with DCs treated with various agents, as evaluated with flow cytometry (n = 3). $CD8^+$ T cells isolated from OT-I mice have T cell receptor specific solely to OVA. (B) Flow cytometric analysis evaluating the percentages of CTV^{low} and IL-2^+ cells among OT-I $CD8^+$ T cells after 3-day coculture with DCs treated with different agents (n = 3). (A, B) *$p < 0.05$ versus PBS, †$p < 0.05$ versus CCM-aCD28, and ‡$p < 0.05$ versus CCM-MPLA. Data are presented as mean ± SD.

3.6. Biodistribution of CCM-MPLA-aCD28

Next, I elucidated a distribution of CCM-MPLA-aCD28 after intradermal injection. CCM–MPLA–aCD28 was prepared from CT26 cells and injected into the skin of CT26 tumor-bearing mice. CCM–MPLA–aCD28 migrated to the adjacent inguinal lymph nodes 24 h after the injection and was scarcely observed in the major organs (3.6.A. and 3.6.B.). A portion of CCM–MPLA–aCD28s was engulfed by dermal DCs and subsequently migrated to the draining lymph nodes. Simultaneously, the other portion of CCM–MPLA–aCD28s appeared to diffuse into the lymph nodes. The inguinal lymph nodes were obtained after 24 h, followed by immunohistochemical (IHC) staining. IHC staining revealed that CCM–MPLA–aCD28 interacts with both DCs and T cells in the lymph nodes (Figure 3.6.C. and 3.6.D).

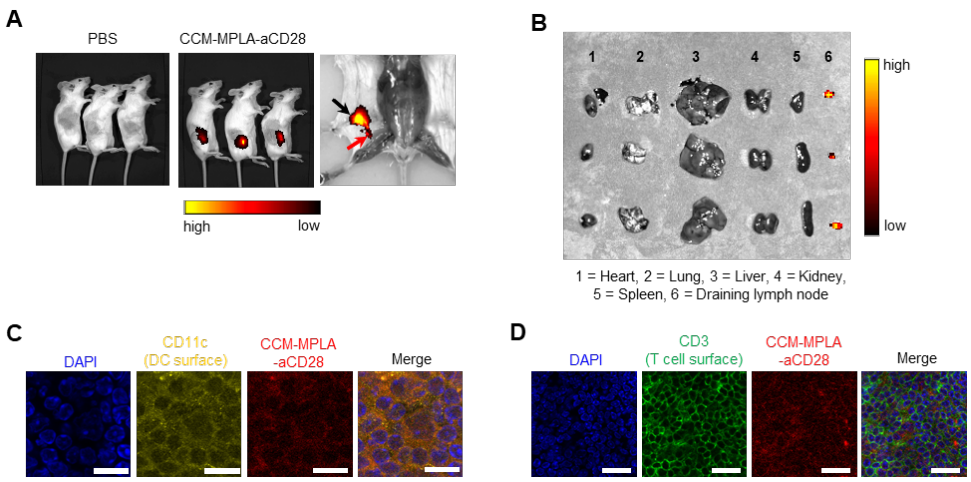

Figure 3.6. Biodistribution of CCM-MPLA-aCD28. (A) *Ex vivo* fluorescence imaging showing localization of intradermally administered CCM–MPLA–aCD28 in the lymph nodes adjacent to the injection site 24 h after the injection. Black arrow: lymph nodes and Red arrow: injection site. (B) *Ex vivo* fluorescence imaging of the major organs (heart, lungs, liver, kidneys, and spleen) and the lymph nodes of mice subcutaneously injected with CCM–MPLA–aCD28 after 24 h. (C, D) Immunohistochemical staining showing the interaction of CCM–MPLA–aCD28 with DCs (C) and T cells (D) in the lymph nodes of mice administered fluorescence-labeled CCM–MPLA–aCD28. (C) Scale bars: 10 μm. (D) scale bars: 20 μm.

3.7. In vivo toxicity of CCM-MPLA-aCD28

The *in vivo* toxicity of CCM–MPLA–aCD28 was also investigated. Five major organs were harvested 5 days after the last injection of CCM–MPLA–aCD28 and stained with hematoxylin and eosin (H&E). These results suggest that CCM–MPLA–aCD28 does not exhibit cytotoxicity toward the organs (Figure 3.7.A). Because nano-sized particles can cause hepatic or renal toxicity,[30,31] we evaluated hepatic [aspartate transaminase (AST) and alanine transferase (ALT)] and renal [blood urea nitrogen (BUN) and creatinine] toxicities. The data revealed no significant differences between the phosphate-buffered saline (PBS)-treated and CCM–MPLA–aCD28-treated mice (Figure 3.7.B.).

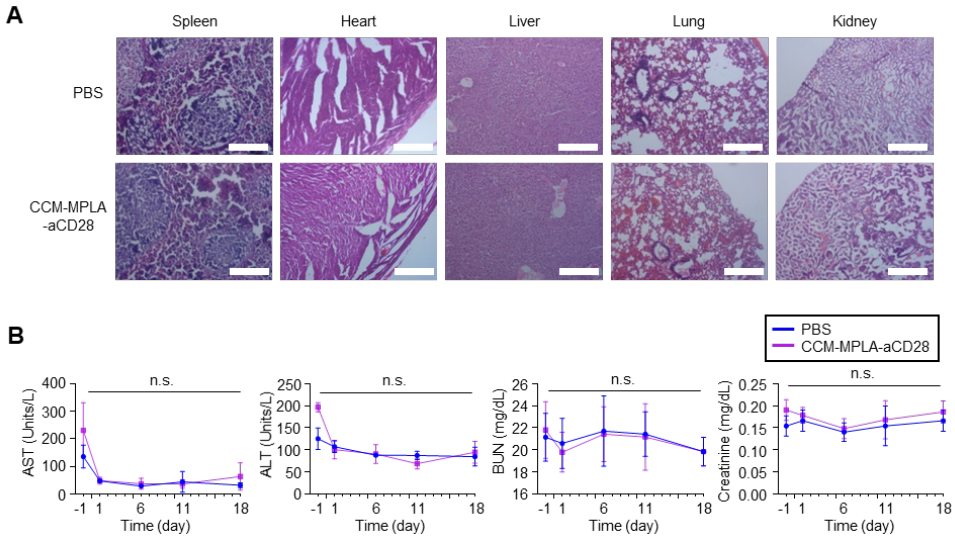

Figure 3.7. In vivo toxicity of CCM-MPLA-aCD28. (A) Hematoxylin and eosin staining images of the major organs of BALB/c mice treated with CCM–MPLA–aCD28. CCM–MPLA–aCD28 does not exhibit any notable toxicity in the major organs. Scale bars: 100 μm. (B) *In vivo* evaluation of hepatic toxicity (AST and ALT) and renal toxicity (BUN and creatinine) of CCM–MPLA–aCD28. CCM–MPLA–aCD28 or PBS was injected at days 0, 5, and 10. Data are presented as mean ± SD. n.s.: not significant.

3.8. The antitumor efficacy of CCM-MPLA-aCD28

I also elucidated whether CCM–MPLA–aCD28 exhibits a higher degree of T cell activation and therapeutic efficacy in tumor-bearing mice than nanovaccines that interact only with DCs (CCM–MPLA) or T cells (CCM–aCD28). Mice bearing CT26 colon cancer cells were vaccinated with various agents three times 5 days after tumor inoculation with a 5-day interval. Compared with all the groups, the tumor growth was suppressed the most in CCM–MPLA–aCD28-treated mice (Figure 3.8.A, B, and D.). In addition, after CCM–MPLA–aCD28 vaccination, CT26-bearing mice had the highest survival rate (Figure 3.8.C.). H&E staining of the tumor tissues revealed that the CCM–MPLA–aCD28 group had the smallest number of cancer cells (Figure 3.8.E.). Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay of tumor tissues revealed that the CCM–MPLA–aCD28 group had the highest number of apoptotic cells (Figure 3.8.F.).

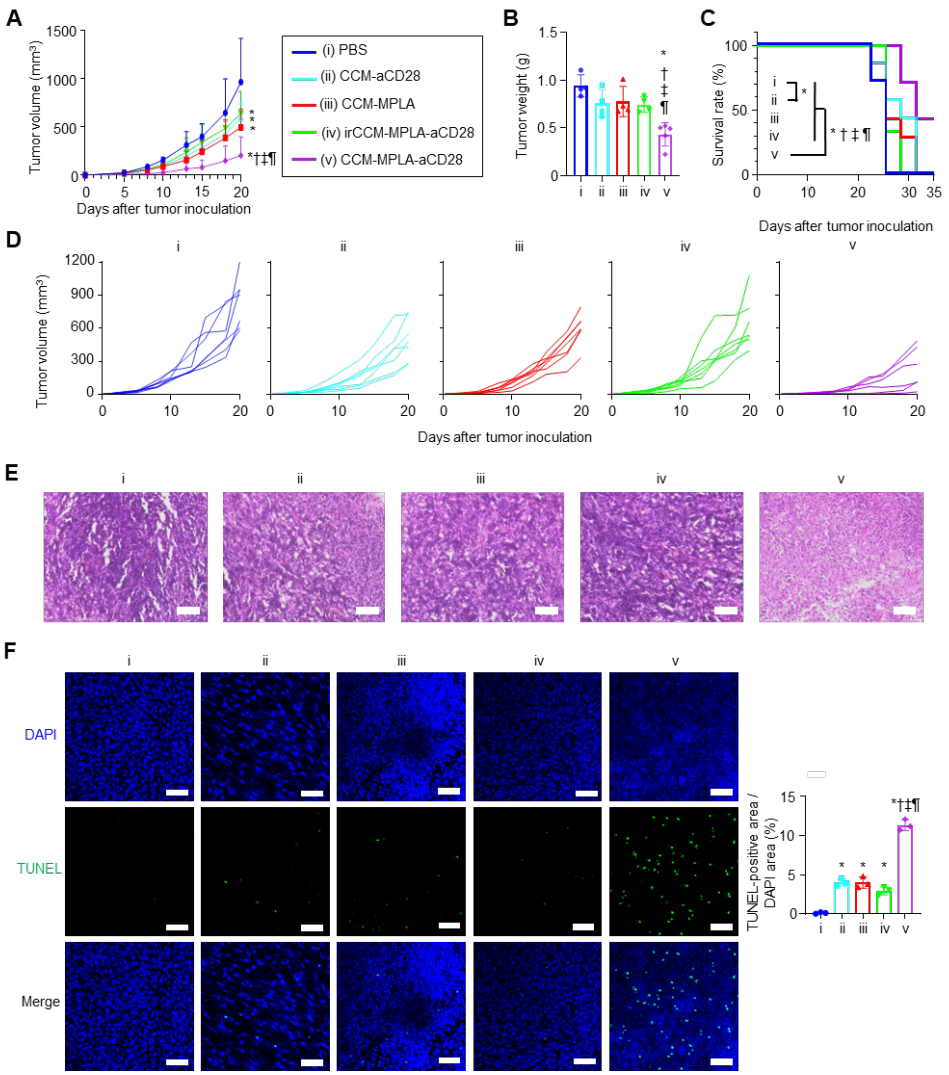

Figure 3.8. The antitumor efficacy of CCM-MPLA-aCD28. CCM was derived from CT26 cells. The irrelevant cancer cell membrane (irCCM) was derived from 4T1 cells. (A) Tumor growth profiles (n = 6–7). (B) Tumor weights on day 20 (n = 4–5). (C) Survival rates of mice (n = 6–7). (D) Individual tumor growth profiles of Figure 6a (n= 6-7). (E) Hematoxylin and eosin (H&E) staining (scale bars: 200 μm) and (F) Terminal deoxynucleotidyl transferase dUTP nick-end labeling assay (scale bars: 25 μm) of tumor tissues on day 20 (n = 3). H&E staining images showed the

lowest number of cancer cells (dark purple) in the group (v). (A-C and F) *$p < 0.05$ versus (i), †$p < 0.05$ versus (ii), ‡$p < 0.05$ versus (iii), and ¶$p < 0.05$ versus (iv). Data are presented as mean ± SD.

3.9. TIL analysis

Analysis of TILs revealed that CCM–MPLA–aCD28 vaccination significantly increased the $CD8^+$ T cell population, leading to an increased ratio of $CD8^+$ to $CD4^+$ T cells in tumor tissues compared with the other treatment groups (Figure 3.9.A.). The expression of cytotoxic cytokines such as granzyme B, IFN-γ, and tumor necrosis factor-alpha (TNF-α) was the highest in $CD8^+$ T cells in the tumor tissues of CCM–MPLA–aCD28-treated mice (Figure 3.9.B.). In addition, the percentage of PD-1^+ $CD8^+$ T cells was the lowest in the tumor tissues of CCM–MPLA–aCD28-treated mice (Figure 3.9.C.), suggesting that CCM–MPLA–aCD28s can prevent $CD8^+$ T cell exhaustion. Taken together, CCM–MPLA–aCD28 vaccination resulted in a higher extent of T cell activation (Figure 3.9.) and higher therapeutic efficacy (Figure 3.8.) in tumor-bearing mice than CCM–MPLA or CCM–aCD28 vaccination.

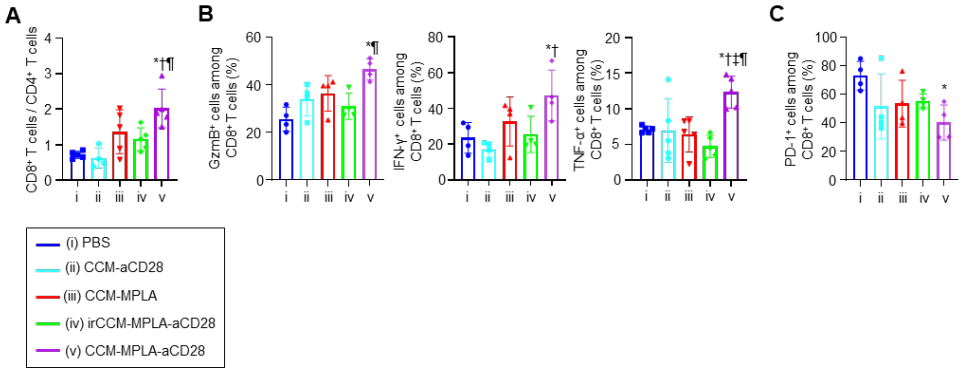

Figure 3.9. TIL analysis of CCM-MPLA-aCD28 injected mice. CCM was derived from CT26 cells. The irrelevant cancer cell membrane (irCCM) was derived from 4T1 cells. (A) The ratio of $CD8^+$ T cells to $CD4^+$ T cells and proportions of (B) antitumoral cytokine (granzyme B, IFN-γ, and TNF-α)-expressing CTLs and (C) T cell exhaustion marker (PD-1)-expressing CTLs in the tumors on day 20, as evaluated using flow cytometry (n = 4–5). (A-C.) *$p < 0.05$ versus (i), †$p < 0.05$ versus (ii), ‡$p < 0.05$ versus (iii), and ¶$p < 0.05$ versus (iv). Data are presented as mean ± SD.

Chapter 4. Conclusion

This study describes a new therapeutic cancer vaccine strategy that can enhance the activation of tumor-specific CTLs by interacting with both DCs and T cells. CCM–MPLA–aCD28, containing cancer cell membrane, MPLA, and aCD28, can activate tumor-specific CTLs. CCM-MPLA-aCD28 activate tumor-specific CTLs via a conventional vaccine mechanism in which tumor antigens are presented to DCs and MLPA facilitates DC maturation, with subsequent activation of tumor-specific CTLs by DCs. Furthermore, CCM–MPLA–aCD28, which provides the MHC class I–tumor antigen complex and aCD28, can use another mechanism to activate tumor-specific CTLs, which is a direct interaction between the vaccine and naïve $CD8^+$ T cells. Therefore, CCM–MPLA–aCD28 exhibits higher antitumor efficacy than nanovaccines that only interact with DCs (CCM–MPLA) or T cells (CCM–aCD28) both in vitro and in vivo.

This study suggests that CCM–MPLA–aCD28 vaccine platform can be used to treat tumors with unidentified tumor antigens. Due to the challenge of identifying immunogenic tumor antigens for individual patients, conventional cancer vaccines are difficult to use in clinical tests. However, CCM–MPLA–aCD28 contains tumor antigens, there is no need to identify the tumor antigens for each patient. CCM–MPLA–aCD28 contains various tumor antigens and may be more effective in tumor inhibition than conventional vaccines that generally use only one type of well-known tumor

antigen peptide because many tumor types are heterogeneous.[32] Furthermore, the CCM–MPLA–aCD28 vaccine platform, which generates antigen-specific CTLs, can be used to treat viral infections or other diseases with well-known antigen peptides.

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Abstract in Korean

Therapeutic Cancer Nanovaccine that Enhances T-cell Responses and Efficacy through Dual Interactions with Dendritic Cells and T Cells

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암 특이적 T 세포를 활성화시키기 위해 주로 수지상 세포와 상호작용하는 기존의 암 백신은 T 세포의 최적화되지 않은 활성화로 인해 충분한 치료 효능을 보여주지 못하는 경우가 많다. 본 연구에서는 이러한 문제점을 해결하기 위해 수지상 세포 및 T 세포 모두와 상호작용하는 치료용 암 나노백신을 개발하였다. 이 나노백신은 암 세포막과 리피드들로 구성되며, monophosphoryl lipid A (MPLA)를 면역보조제로 포함하는 리포좀 유사 나노입자 (CCM-MPLA)를 형성한다. 암 특이적 T 세포와의 직접적인 상호작용을 달성하기 위해, CCM-MPLA에 CD28 항체 (aCD28)를 결합하여 CCM-MPLA-aCD28을 생성하였다. 이 나노백신은 수지상 세포의 유무와 무관하게 암 특이적 $CD8^+$ T 세포의 치료 효능을 증가시킬 수 있다. CCM-MPLA-aCD28은 DC (CCM-MPLA) 또는 T 세포 (CCM-aCD28)와 상호작용하는

기존의 나노백신들과 비교해 더 많은 암 특이적 $CD8^+$ T 세포의 활성을 유도하며, 마우스 모델에서 더 높은 항암효과를 보였다. 또한, CCM-MPLA와 CCM-aCD28 사이 T 세포 활성화 정도 및 치료 효능에 유의미한 차이가 발견되지 않았다. 이 방법은 자가 암 세포막을 포함하는 효과적 개인 맞춤 암 백신 개발에 기여할 수 있을 것이다.

Keyword : 암 면역치료, 암 백신, T 세포, 수지상 세포, 암 세포막

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