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Synergistic STING activation by PC7A nanovaccine and ionizing radiation improves cancer immunotherapy

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ABSTRACT

Solid cancers are able to escape immune surveillance and are resistant to current treatment in immunotherapy. Recent evidence indicates the critical role of the stimulator of interferon genes (STING) pathway in antitumor immunity. STING-targeted activation is extensively investigated as a new strategy for cancer therapy. Previously, we reported a safe and efficacious STING-activating nanovaccine to boost systemic tumor-specific T cell responses in multiple tumor models. Local radiotherapy has been reported to not only reduce tumor burden but also enhance local antitumor immunity in a STING-dependent manner. In this study, we demonstrate that combination of these two modalities leads to a synergistic response with long-term regression of large established tumors in two mouse tumor models. The percentage of $CD8^+$ T cells increased significantly in primary tumors after combination therapy. Mechanistically, the augmented T cell responses of radiotherapy and nanovaccine is STING pathway dependent. Furthermore, nanovaccine synergizes with radiotherapy to achieve a better therapeutic effect in distal tumors. These findings suggest that combination of local radiotherapy with systemic PC7A nanovaccine offers a useful strategy to improve the therapeutic outcome of late stage solid cancers.

1. Introduction

Stimulation of innate immune pathways plays an important role in T cell production and tumor infiltration [1]. Among various innate pathways, stimulator of interferon genes (STING) is emerging as a unique mediator protein for host defense. STING exists in many cell types, including all antigen-presenting cells, epithelial cells, endothelial cells, and fibroblasts [2], which provide a broad platform for effective innate stimulation. Activation of the STING pathway upregulates the transcription of genes that encode type I interferons (IFNs) and proinflammatory cytokines and chemokines. STING activation in tumor tissues can lead to accumulation and infiltration of CD8+ T cells. Recent studies show after tumor cell inoculation in STING-deficient mice, tumors grew more rapidly than in wild-type or TRIF-deficient mice. $CD8^+$ T cells against tumors were also defective in mice lacking STING, but not in those lacking Toll like receptor, myeloid differentiation primary response 88 (MyD88) or mitochondrial antiviral signaling protein (MAVS), suggesting the essential role of STING in antitumor immunity [3].

We previously reported a synthetic polymeric nanoparticle, PC7A NP, which generated a robust cancer-specific T cell response with low systemic cytokine expression [4]. Antigen-loaded PC7A NP was systemically administered by subcutaneous injection to target the lymphoid organs. The nanoparticle formulation (20–30 nm in diameter) allowed efficient cytosolic delivery of tumor antigens to the dendritic cells inside draining lymph nodes, while stimulating the innate pathway for T cell activation. T cell activation is exclusively dependent on STING, but not on TLR or MAVS pathways. The PC7A nanovaccine

Abbreviations: STING, stimulator of interferon genes; IFN, interferon; RT, radiotherapy; LN, lymph node; APC, antigen-presenting cell; CTL, cytotoxic T lymphocyte; DC, dendritic cell; PBS, phosphate-buffered saline

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illustrated efficacious anti-tumor response in multiple mouse tumor models, including B16 melanoma, MC38 colon carcinoma, and TC-1 cervical tumors. Despite the therapeutic promise, tumor resistance to PC7A nanovaccine is observed in established tumors (e.g., > 100 mm^3). Combination of other therapeutic modalities with PC7A nanovaccine may prove necessary in the eradication of large, immunosuppresive tumors.

Radiotherapy (RT) is widely used in the treatment of various solid tumors. Increasing evidence has shown that local radiation not only reduce tumor burden [5,6], but also augment adaptive T cell response against tumors [7,8]. Deng et al. reported that ionizing radiation produces a type I IFN-dependent antitumor response via the STING pathway. The DNA of dying irradiated cancer cells are found in the cytoplasm of dendritic cells, which is responsible for the activation of the cGAS-STING-type I IFN pathway [9]. Direct activation of STING by intra-tumoral injection of STING agonists also led to potent immune responses and systemic tumor regression [10]. Therefore, targeting the STING pathway in tumors offers an effective strategy to enhance adaptive antitumor immunity.

In this study, we investigated the synergy between the systemic cancer-specific T cell response initiated by STING-activating PC7A nanovaccine, and local STING activation by ionizing radiation for increased T cell infiltration in tumors. The combined STING activation strategy produced a synergistic therapeutic outcome against large, established tumors compared to either treatment alone. STING-deficient mice treated with the same strategy showed significantly less $CD8^+$ T cells both systemically and locally in the tumor. In addition to the response seen in the primary irradiated tumors, we also observed an abscopal effect from this combination, which is rarely found in patients treated with radiotherapy alone. These data suggest combining PC7A nanovaccine with local radiation offers a useful strategy in treating both primary and metastatic cancers.

2. Materials and methods

2.1. Chemicals

Antigenic peptides $E7_{43–62}$ (GQAEPDRAHYNIVTFCCKCD), $OVA_{257–280}$ (SIINFEKLTEWTSS NVMEERKIKV), were synthesized by Biomatik. Fetal bovine serum, penicillin streptomycin, and cell culture media were obtained from Invitrogen Inc. (OR, USA). Amicon ultra-15 centrifugal filter tubes (MWCO = 100 K) were from Millipore. Other solvents and reagents were purchased from Sigma-Aldrich or Fisher Scientific Inc.

2.2. Syntheses and preparation of PC7A micelle nanoparticles

2-(Hexamethyleneimino) ethyl methacrylate (C7A-MA) monomer was synthesized following previous procedures [11,12]. PEG-*b*-PC7A copolymer was synthesized by atom transfer radical polymerization (ATRP) method [13]. Micelles were prepared following a solvent evaporation method [12]. Briefly, 10 mg of PEG-*b*-PC7A copolymer was first dissolved in 1 mL methanol and then added into 4 mL distilled water dropwise under sonication. The mixture was filtered 4 times to remove methanol using the micro-ultrafiltration system (MW = 100 KD). Then distilled water was added to adjust the polymer concentration to 10 mg/mL as a stock solution. After micelle formation, the nanoparticles were characterized by dynamic light scattering (DLS, Malvern ZetaSizer model) for hydrodynamic diameter (Dh) measurement.

2.3. Animals and cells

All animal procedures were performed with ethical compliance and approval by the Institutional Animal Care and Use Committee at the University of Texas Southwestern Medical Center. Female C57BL/6 mice (6–8 weeks) were obtained from the University of Texas Southwestern breeding core. $STING^{mut/mut}$ mice were purchased from the Jackson laboratory. B16-OVA cells were kindly provided by Dr. Patrick Hwu at MD Anderson Cancer Center and TC-1 cells by Dr. T. C. Wu at John Hopkins University. Both cell lines were routinely tested using mycoplasma contamination kit (R&D). Cells were cultured in DMEM medium (10% fetal bovine serum, 100 U/mL penicillin G sodium and 100 μg/mL streptomycin (Pen/Strep), non-essential amino acids, and 20 μM β-mercaptoethanol (β-ME)) at 37 °C in 5% CO_2 and the normal level of O_2.

2.4. Tumor inoculation and treatment

Six to eight week old mice (n = 5–10 for each group) were injected subcutaneously with B16-OVA (1.5×10^5), or TC-1 cells (1.5×10^5) into the right flank of mice. Tumors were treated by local radiation as described previously [9]. Animals were immunized with subcutaneous injection at the tail base (0.5 μg per antigen peptide, PC7A NP 30 μg). The tumor growth was subsequently measured twice a week using a digital caliper and tumor size was calculated as $0.5 \times \text{length} \times \text{width}^2$ by blinded investigators. Mice were sacrificed when tumor size reached 1500 mm^3. For cell depletion experiments, 250 μg anti-CD8 (clone 2.43; Bio-X Cell) or anti-NK-1.1 (clone PK136; Bio-X Cell) was administered four times by i.p. injection every 3 days per mouse.

2.5. Flow cytometry analysis

For $CD8^+$ T cell and $Tetramer^+$ cell analyses, tumor tissues were digested by 1 mg/mL collagenase IV (Sigma-Aldrich) and 0.2 mg/mL DNase I (Sigma-Aldrich) for 45 min at 37 °C. Cells were then stained with anti-CD16/CD32 (Biolegend, Cat#: 101301, clone: 93), anti-CD45.2-APC (Biolegend, Cat#:109814, clone:104), anti-mouse CD3-Alexa Fluor® 488 (Biolegend, Cat#: 100210, clone: 17A2), anti-mouse CD4-Brilliant Violet 785(Biolegend, Cat#: 100551, clone: RM4–5), anti-CD8-FITC (Thermo Fisher Scientific, Cat#: MA5–16759, clone: KT15), and Tetramer/PE – H−$2D^b$ HPV 16 E7 (RAHYNIVTF) (MBL). Flow data were collected on a BD™ LSR II flow cytometer or CytoFLEX (Beckman Coulter, Inc) and analyzed with FlowJo (Tree Star Inc., Ashland, OR) or CytExpert (Beckman Coulter, Inc) software.

2.6. Histology and immunohistochemistry (IHC) of tumor tissues

Tissues were fixed with 4% paraformaldehyde for 2–3 days and sent to university histology core for paraffin sectioning. Paraffin sections were deparaffinized and rehydrated with xylene and serial dilutions of ethanol followed by antigen retrieval with 1× Tris-EDTA in 10% glycerol buffer (pH 9.0). Sections were blocked by 5% bovine serum albumin in Tris Buffer Solution plus Tween (TBST) and incubated with primary rabbit monoclonal anti-mouse CD8 (11000) (98941S, Cell Signaling Technology) in blocking solution at 4 °C overnight. Then slides were washed and incubated for 45 min with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgGs (MP-7451-15, Vector Laboratories), color was developed with 3,3′ Diaminobenzidine (DAB) substrate and the slides were finally counterstained with hematoxylin.

2.7. Statistical analysis

Based on pilot immunization and tumor treatment studies, we used group sizes of 3–6 animals/group for immunogenicity measurements and 5 animals/group for tumor therapy experiments. Statistical analysis was performed using Microsoft Excel and Prism 5.0 (GraphPad). Data are expressed as means ± s.e.m. Data were analyzed by Student's *t*-test. Variance similarity test (*f*-test) was performed before *t*-test. All *t*-tests were one-tailed and unpaired, and were considered statistically significant if $p < .05$ (*, $p < .05$; **, $p < .01$; ***, $p < .001$ unless otherwise indicated). The survival rates of the two groups were analyzed using a log-rank test and were considered statistically significant

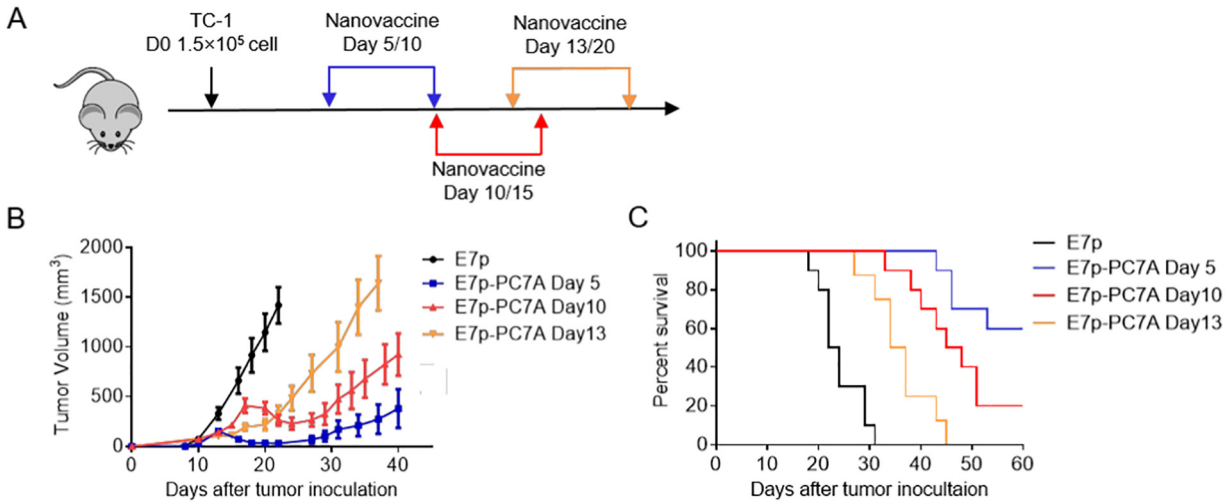

Fig. 1. Effect of PC7A nanovaccine on the size and stage of TC-1 tumor models. (A) Scheme of the designed treatments in TC-1 tumor model. Control group were treated with E7 peptide alone (0.5 μg per mouse) twice on day 5 and day 10 after tumor inoculation. Blue arrow shows one group of mice received vaccination (0.5 μg $E7_{43–62}$ peptide plus 30 μg PC7A NP per mouse) on day 5 and 10 after tumor inoculation. Red arrow shows vaccination on day 10 and 15 after tumor inoculation, and finally orange arrow shows vaccination on day 13 and 20. Tumor growth inhibition (B) and long-term survival data (C) in C57BL/6 mice were analyzed after tumor inoculation with TC-1 tumor cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

if $p < .05$.

3. Results

3.1. Established tumors develop resistance to PC7A nanovaccine

We previously demonstrated that STING-activating PC7A nanovaccine generated potent tumor-specific T cell responses in several mouse tumor models [4]. However, the dependence of antitumor efficacy of the vaccine therapy on the different size and stage of tumor development is not clear. To address this question, we immunized mice at several time points after TC-1 tumor cell inoculation. When mice were immunized with $E7_{43–62}$ –PC7A vaccine 5 days after tumor cell inoculation, 70% of mice showed complete regression 2 weeks after vaccination, and 60% mice showed tumor free survival 60 days after tumor inoculation (Fig. 1, Supplementary Fig. 1). In contrast, when mice were immunized on day 10 after tumor cell inoculation, the tumors were well-established (about 100–200 mm^3). The tumors showed some shrinkage a week after the first vaccination, but the effect was not adequate to reject the tumors, which finally recurred a month later. When mice were immunized 13 days after tumor cell inoculation, this group showed continuous tumor growth and shorter life span compared to the other two groups. At the same time, $CD8^+$ T cells in tumors at different time points after tumor inoculation were examined. Results showed that larger tumors contained lower percentage of T cells (Supplementary Fig. 2A), and PD-L1 was expressed on tumor cells and several subtypes of myeloid cells over isotype control [4].These data illustrate that larger tumors develop immunosuppressive mechanism and become resistant to nanovaccine therapy. From our previous report, after injection of fluorescence labeled nanoparticles, antigen presenting cells at draining lymph nodes and injection site especially DCs were the major cell population that took up PC7A NPs and subsequently activated STING-type I IFN pathway [4]. During this process, no fluorescence signal was detected in tumors (Supplementary Fig. 2B), which suggests the antitumor efficacy was mostly due to T cell activation in lymphoid organs. In cell depletion assay, $CD8^+$ T cell depletion abolished majority of nanovaccine-induced anti-tumor efficacy, but not NK cell depletion (Supplementary Fig. 2C, D). These data suggest that T cells induced by nanovaccine in the lymphatic system played a major role in tumor growth inhibition.

3.2. Radiation synergizes with nanovaccine to effectively control established tumors

To investigate whether local radiation can help overcome tumor resistance to PC7A vaccine, we employed established TC-1 tumors in C57BL/6 mice with tumor size reaching ~200 mm^3 (in about 13 days after inoculation). Based on previous reports, T lymphocytes are highly sensitive to ionizing radiation and can be cleared rapidly from the radiation site [14,15], so in the combination group, mice first received a single dose of 20 Gy local radiation, followed by subcutaneous injection of PC7A nanovaccine, with a boost 7 days post initial vaccination (Fig. 2A). Nanovaccine alone and radiation alone were used as controls to assess the effect of monotherapy. The result showed that radiation alone and nanovacccine alone deterred the tumor growth marginally compared with the E7 peptide only control, but were unable to suppress eventual tumor growth. In contrast, combination of nanovaccine and radiation therapy showed significant therapeutic synergy over either monotherapy alone, as 50% of mice were tumor-free 60 days after tumor inoculation (Fig. 2B–C, Supplementary Fig. 3A–D). Tumor-free mice were rechallenged with 1×10^6 TC-1 tumor cells (Supplementary Fig. 3E), and animals showed long-term memory to reject the transplanted tumors, whereas tumors grew robustly in naïve mice.

This synergistic effect was also evaluated in B16-OVA melanoma tumor model. After the tumor size reached 70 mm^3 (about 10 days after inoculation), the mice received a single dose of 20 Gy local radiation. Nanovaccine containing OVA peptide was subcutaneously administered immediately post-radiation, with two boost shots 5 and 10 days post initial vaccine treatment (Fig. 2D). This combination also showed obvious synergy effect over single therapy, as 40% mice were tumor-free 60 days after tumor inoculation (Fig. 2E and F). These results demonstrate great therapeutic synergy in systemic PC7A vaccine and local radiation therapy.

3.3. STING pathway is required for the therapeutic synergy of combination therapy

Since either PC7A nanovaccine or local radiation were shown to depend on STING pathway for antitumor immunity, we sought to determine the role of STING in the combination strategy. We first determined the ratio of tumor-infiltrating T cells over cancer cells from various treatment groups. Tumor tissues were removed for analysis

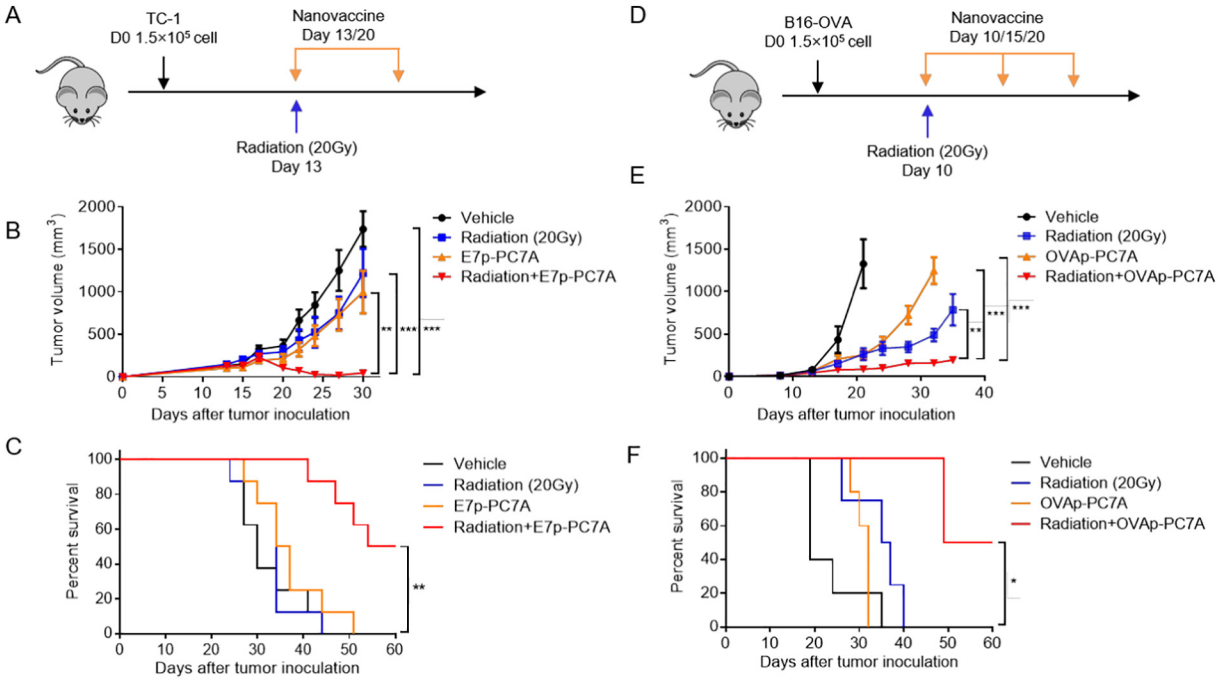

Fig. 2. Synergistic effect of PC7A nanovaccine and radiation therapy in established tumor models. (A) Scheme of treatment regimens in the TC-1 tumor model. (B) Tumor growth inhibition and (C) long-term survival data in C57BL/6 mice were analyzed after tumor inoculation with TC-1 tumor cells. (D) Scheme of treatment regimens in the B16-OVA tumor model. (E) Tumor growth inhibition and (F) survival data in C57BL/6 mice were analyzed after tumor inoculation with B16-OVA tumor cells. $***P < .001$, $**P < .01$, $*P < .05$.

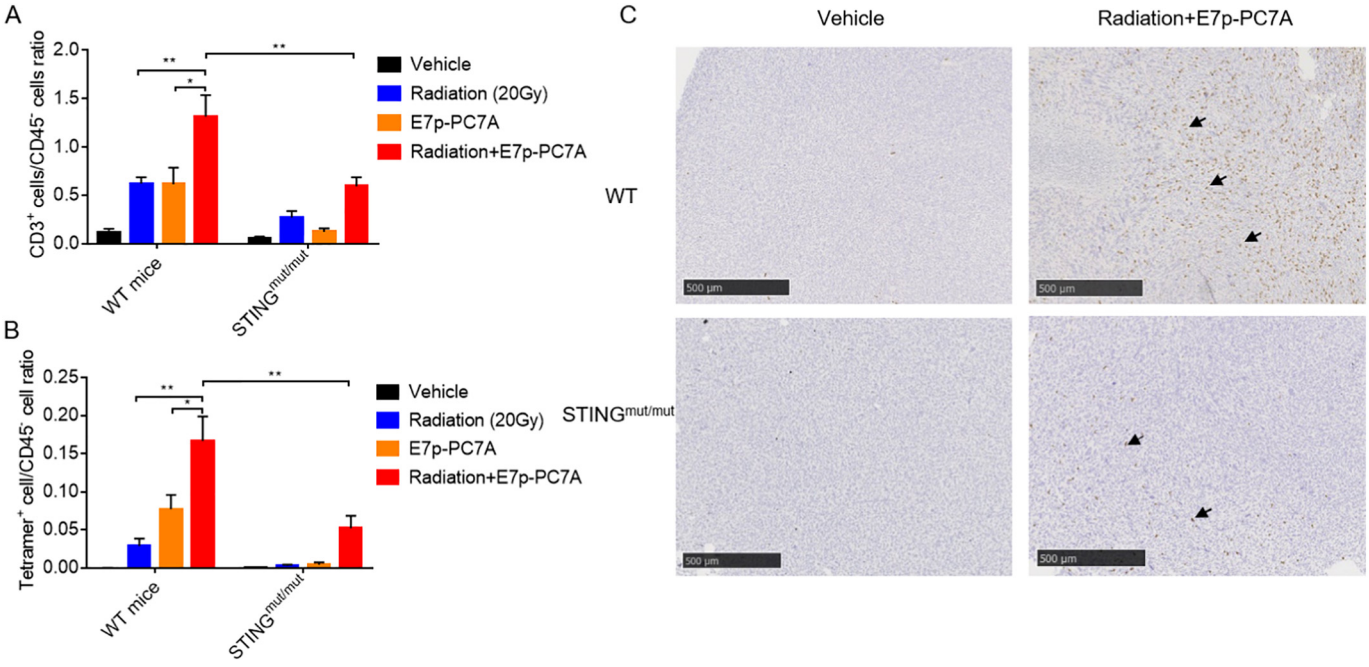

Fig. 3. STING pathway is required for tumor-specific T cell response of combination therapy. (A) Percentage of lymphocytes ($CD3^+$) in the tumor tissues of the wild type (WT) mice or STING mutant mice. (B) Percentage of E7-epitope specific T lymphocytes ($CD8^+Tetramer^+$) to tumor cell ratio of wild type or STING mutant mice. $**P < .01$, $*P < .05$. (C) Immunohistochemical analysis of $CD8^+$ cells in tumor tissues in wild type and STING mutant mice. Arrows showed staining for $CD8^+$ lymphocytes.

5 days after the second vaccination in mice inoculated with the TC-1 tumors. Tumor tissues were dissociated into single cells by collagenase treatment. Flow cytometry was then utilized to detect the percentage of infiltrating T cells (Supplementary Fig. 4). The results show that compared to non-treated WT mice, both radiation alone and vaccine alone treatment increased tumor infiltrating T cells ($CD45^+CD3^+$cells) and antigen-specific $CD8^+$ T cells (E7 $tetramer^+$ $CD8^+CD3^+$cells). Moreover, combination of radiation and vaccine treatment results in significant increase of both T cell populations over single arm controls, demonstrating clear therapeutic synergy (Fig. 3A–B). T cell infiltration in tumors correlated with anti-tumor efficacy (Fig. 2B–C).

In STING mutant mice, results show that majority of the T cell accumulation and infiltration especially tumor specific $CD8^+$ T cells were abolished compared to radiation alone and vaccine alone groups from

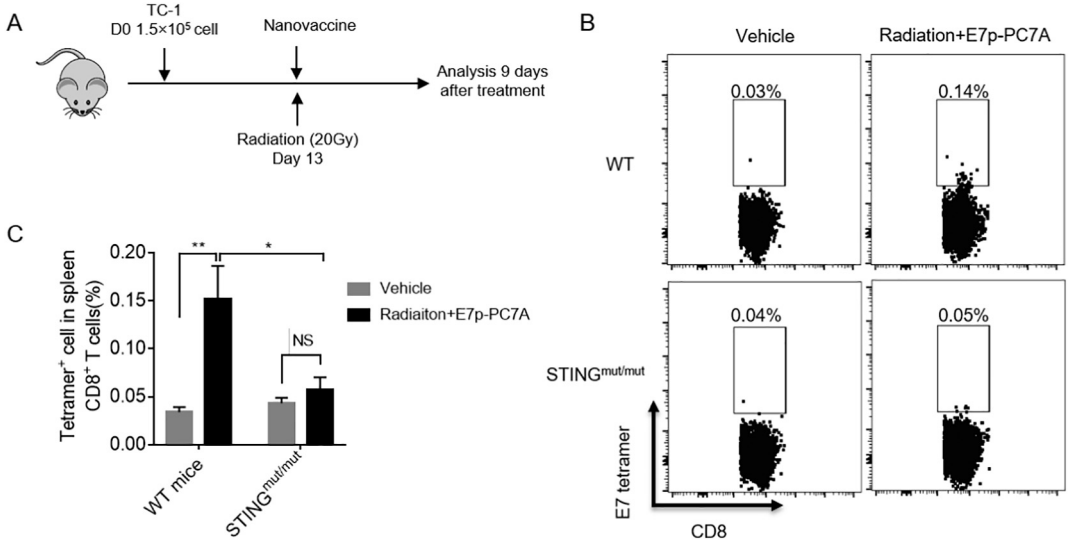

Fig. 4. STING pathway is required for systemic T cell activation of combination therapy. (A) Scheme of treatment regimens in the TC-1 tumor model. (B) Representative flow dot plots of H-−2D^b HPV16 E7 (RAHYNIVTF) tetramer staining of CD8$^+$ T cells in the spleen of wild type and STING mutant mice. (C) Percentage of E7-specific CD8$^+$ T cells show significantly increased systemic T cell response in wild type over STING mutant mice. $^{**}P < .01$, $^{*}P < .05$. NS, not significant.

the wild type animals (Fig. 3A–B), which is consistent with our previous reports [4,9]. For the combination therapy group, compared to wild type mice, STING mutant mice showed significantly decreased lymphocytes in tumor after treatments ($P < .01$). Furthermore, we used immunohistochemistry assay to detect the percentage of CD8$^+$ T cell infiltration into the tumors (Fig. 4C). In wild type mice, a higher percentage of tumor-infiltrating CD8$^+$ T cells were detected compared to STING mutant mice after combination therapy. To further corroborate these findings, we evaluated the systemic T cell activation 9 days after radiation and the first dose of vaccine in both wild type and STING mutant mice (Fig. 4). Spleens were removed and dissociated into single cells for flow cytometry analysis (Supplementary Fig. 5). The treated group showed distinctive increase of E7-specific T cells in CD8$^+$ T cell population compared to the untreated group and STING mutant group (Fig. 4B–C). These experiments demonstrate that STING pathway is required for both elevated systemic T cell response and local tumor infiltration in the combination therapy.

3.4. Local radiation synergizes with PC7A nanovaccine to control distal tumors

Ionizing radiation has been reported to reduce tumor growth outside the treatment field in rare clinical cases, referred to as the abscopal effect [16,17]. Enhancement of the abscopal effect has great promise in treatment of patients with metastatic tumors. Historically, preclinical evidence has supported the notion that distal tumor (metastatic tumor) regression as a result of radiation is immune-mediated [18–20]. In this study, we used the dual TC-1 tumor model to test the abscopal effect. Primary tumors were first initiated by subcutaneous injection of cells (1.5×10^5 cells) on the left flanks of mice, and 4 days later, distal tumors (2×10^4 cells) were introduced on the right flanks (Fig. 5A).

In the untreated control group, distal tumors grew both later and smaller than the primary tumors (Supplementary Fig. 6A), resembling metastatic tumor nodules in patients. When primary tumor size reached ~200 mm^3 (in about 13 days), mice received a single dose of 20 Gy local radiation on primary side and PC7A vaccine subcutaneously. We measured tumor growth on both the primary and distal tumors. Results show radiation alone was effective at inhibiting the growth of the irradiated primary tumor (Fig. 5B), but it had no effect on distal tumors compared to non-treated group. PC7A nanovaccine alone showed increased inhibitory effect on distal tumors over the non-treated control, and combination of both nanovaccine and radiation achieved additional growth inhibition in distal tumors (Fig. 5C–D, Supplementary Fig. 6B). These results illustrate radiation alone failed to prime a durable immune response to attack distal TC-1 tumors, but combination with STING vaccine led to a significant improvement in systemic T cell response against distal tumors.

4. Discussion

Therapeutic vaccines are designed to harness the immune system to induce potent tumor-specific cytotoxic T cells for cancer immunotherapy. New trends in therapeutic cancer vaccines focus on the mode of antigen delivery and the type of immune-stimulating adjuvants [21–23]. Our PC7A nanovaccine allowed stable antigen loading within a small size confinement (< 50 nm) that facilitates antigen delivery to the lymph nodes. So this nanovaccine platform can be rapidly adopted to incorporate many existing tumor-associated antigens as well as tumor neoantigens. Equally important, early endosomal release of tumor antigens into the cytosol avoids lysosomal degradation, leading to increased antigen cross-presentation on the cell surface. Uniquely, this synthetic nanoparticle itself stimulated innate cellular immunity through the STING-type I IFN pathway, which induced a long term antitumor response. Compared to several established adjuvants (CpG, polyI:C and Alum), PC7A NP was able to induce better T cell response and anti-tumor efficacy with lower systemic cytokine expressions[4]. In large, established tumors, this vaccine showed decreased antitumor efficacy. Historically, clinical trial outcomes of many cancer vaccines have also been disappointing [24–27] with similar limitations in treating late stage tumors. Therefore, developing strategies to reduce tumor burden and overcome resistance of established tumors to cancer vaccines has the potential to improve the clinical result of therapeutic vaccines.

Tumor radiation can rapidly reduce tumor burden without directly suppressing vaccine-induced systemic T cell responses. Increasing evidence shows that radiation can augment adaptive T cell responses to tumors [7,8]. First, radiation can damage the tumor tissue and reorganize tumor-associated blood vessels, which presumably allows for better T cell or cytokine penetration [28,29]. Second, it has been shown that the therapeutic effect of ablative radiation therapy depends largely on CD8$^+$ T cells, as radiation increases T cell priming [30]. Furthermore, local radiation induces antigen release and cross-presentation

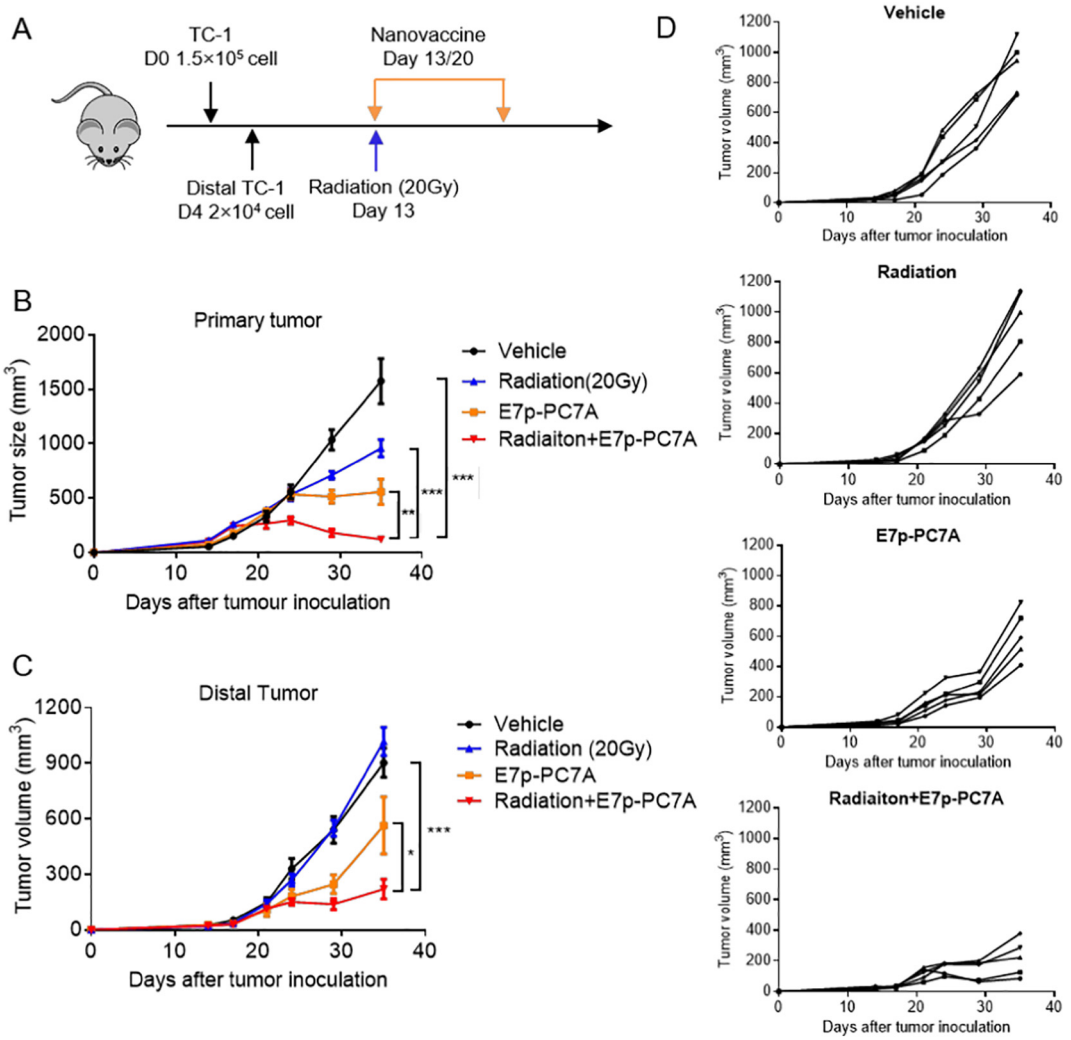

Fig. 5. Synergistic effect of PC7A nanovaccine and radiation therapy on distal TC-1 tumor growth. (A) Schematic design of the experiments. (B) Tumor growth curve of primary tumors after different treatments. (C) Distal tumor growth in C57BL/6 mice was analyzed for different treatment groups. (D) Individual tumor growth curves for control group, radiation alone, E7p-PC7A NP alone and E7p-PC7A NP combined with radiation. Synergistic outcome was observed in growth inhibition of distal tumors by combination therapy. $^{***}P < .001$, $^{**}P < .01$, $^{*}P < .05$.

mainly through STING-Type I IFNs pathway [20]. Previous reports showed that only antigens released by dead tumor cells were not sufficient to stimulate T cell activation. Under non-inflammatory conditions, these dead cells failed to induce antitumor immune responses [31]. Radiation not only induces antigens' release from tumor cells, but also triggers innate sensing, stimulates and attracts antigen-presenting cells (APCs) to uptake and present tumor antigens, as well as increases expression of co-stimulators, finally activates tumor specific T cell response. Among different innate sensing pathways that modulate tumor inflammation, it has been shown that STING-type I IFNs were required for radiation-induced inflammation and subsequent adaptive immune responses [3,9]. Due to the increasing recognition of the importance of STING pathway in T cell priming and anti-tumor effects, we combined our nanovaccine with local ionizing radiation to synergize STING function in both the lymphatic system and tumor site. This combination showed an obvious synergy effect in established tumor models. In STING mutant mice, the T cell response produced by this combination was markedly decreased, indicating that the STING pathway and subsequent pro-inflammatory cytokines expressed in the tumor are crucial for allowing T cells to home to tumors and attack. Our data show encouraging antitumor efficacy in relatively large tumors by combining radiation and vaccine therapy. Kelly et al. reported using a combination of four immunotherapeutic agents to eliminate large tumor burdens [32]. Inclusion of additional agents that target independent immune suppressive mechanisms (e.g., anti-PD-1 or PD-L1) may be beneficial to augment the antitumor response in the current regimen.

In this study, we further investigated the combination therapy on distal tumors, and observed that distal tumors displayed a similar synergistic response to the combined treatments as the primary tumors, but were more resistant. In several mice, while the primary large tumors showed obvious regression after treatment, the distal tumor still persisted and eventually grew (Fig. 5 and Supplementary Fig. 6), as reported in similar studies [33]. One possible explanation for this diminished response might be the intact immunosuppressive environment of the distal tumors (i.e., without radiation-induced inflammation). Another possibility is the attraction of more circulating T cells to the primary tumor site, which resulted in fewer T cells accumulating in the distal tumor. Further work is necessary to elucidate the mechanism of vaccine resistance in distal tumors to achieve optimal abscopal effect.

5. Conclusions

We previously reported a STING-activating nanovaccine to elicit a robust T cell response against multiple tumor types. In this study, we combined the nanovaccine with radiation therapy to treat large, established solid tumors. The combination regimen showed a synergistic

effect in both established and distal tumors. Mechanistically, local radiation effectively reduced the tumor burden and reverted immunosuppressive environment through the activation of STING pathway. The spatial orchestration of STING activation, systemically through the PC7A nanovaccine and locally by tumor radiation, resulted in significantly improved tumor growth inhibition and long-term survival in TC-1 and B16-OVA tumor-bearing mice. The antitumor efficacy was greatly diminished in STING mutant mice. Results from this study indicate the importance of synergizing STING activation of the lymphoid organs and solid tumors for cancer immunotherapy.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jconrel.2019.02.036.

References

[1] O. Takeuchi, S. Akira, Pattern recognition receptors and inflammation, Cell 140 (2010) 805–820.
[2] X.D. Li, J. Wu, D. Gao, H. Wang, L. Sun, Z.J. Chen, Pivotal roles of cGAS-cGAMP signaling in antiviral defense and immune adjuvant effects, Science 341 (2013) 1390–1394.
[3] S.R. Woo, M.B. Fuertes, L. Corrales, S. Spranger, M.J. Furdyna, M.Y. Leung, R. Duggan, Y. Wang, G.N. Barber, K.A. Fitzgerald, et al., STING-dependent cytosolic DNA sensing mediates innate immune recognition of immunogenic tumors, Immunity 41 (2014) 830–842.
[4] M. Luo, H. Wang, Z. Wang, H. Cai, Z. Lu, Y. Li, M. Du, G. Huang, C. Wang, X. Chen, et al., A STING-activating nanovaccine for cancer immunotherapy, Nat. Nanotechnol. 12 (2017) 648–654.
[5] S.L. Liauw, P.P. Connell, R.R. Weichselbaum, New paradigms and future challenges in radiation oncology: an update of biological targets and technology, Sci. Transl. Med. 5 (2013) 173sr172.
[6] A.C. Begg, F.A. Stewart, C. Vens, Strategies to improve radiotherapy with targeted drugs, Nat. Rev. Cancer 11 (2011) 239–253.
[7] L. Deng, H. Liang, B. Burnette, M. Beckett, T. Darga, R.R. Weichselbaum, Y.X. Fu, Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice, J. Clin. Invest. 124 (2014) 687–695.
[8] M.M. Wattenberg, A. Fahim, M.M. Ahmed, J.W. Hodge, Unlocking the combination: potentiation of radiation-induced antitumor responses with immunotherapy, Radiat. Res. 182 (2014) 126–138.
[9] L. Deng, H. Liang, M. Xu, X. Yang, B. Burnette, A. Arina, X.D. Li, H. Mauceri, M. Beckett, T. Darga, et al., STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors, Immunity 41 (2014) 843–852.
[10] L. Corrales, L.H. Glickman, S.M. McWhirter, D.B. Kanne, K.E. Sivick, G.E. Katibah, S.R. Woo, E. Lemmens, T. Banda, J.J. Leong, et al., Direct activation of STING in the tumor microenvironment leads to potent and systemic tumor regression and immunity, Cell Rep. 11 (2015) 1018–1030.
[11] K. Zhou, Y. Wang, X. Huang, K. Luby-Phelps, B.D. Sumer, J. Gao, Tunable, ultrasensitive pH-responsive nanoparticles targeting specific endocytic organelles in living cells, Angew. Chem. 50 (2011) 6109–6114.
[12] K. Zhou, H. Liu, S. Zhang, X. Huang, Y. Wang, G. Huang, B.D. Sumer, J. Gao, Multicolored pH-tunable and activatable fluorescence nanoplatform responsive to physiologic pH stimuli, J. Am. Chem. Soc. 134 (2012) 7803–7811.
[13] N.V. Tsarevsky, K. Matyjaszewski, “Green” atom transfer radical polymerization: from process design to preparation of well-defined environmentally friendly polymeric materials, Chem. Rev. 107 (2007) 2270–2299.
[14] R.E. Vatner, B.T. Cooper, C. Vanpouille-Box, S. Demaria, S.C. Formenti, Combinations of immunotherapy and radiation in cancer therapy, Front. Oncol. 4 (2014) 325.
[15] E.M. Rosen, S. Fan, S. Rockwell, I.D. Goldberg, The molecular and cellular basis of radiosensitivity: implications for understanding how normal tissues and tumors respond to therapeutic radiation, Cancer Investig. 17 (1999) 56–72.
[16] S. Siva, J. Callahan, M.P. MacManus, O. Martin, R.J. Hicks, D.L. Ball, Abscopal [corrected] effects after conventional and stereotactic lung irradiation of non-small-cell lung cancer, J. Thorac. Oncol. 8 (2013) e71–e72.
[17] J. Ng, T. Dai, Radiation therapy and the abscopal effect: a concept comes of age, Ann. Transl. Med. 4 (2016) 118.
[18] K.E. Hellstrom, I. Hellstrom, J.A. Kant, J.D. Tamerius, Regression and inhibition of sarcoma growth by interference with a radiosensitive T-cell population, J. Exp. Med. 148 (1978) 799–804.
[19] R.J. North, Radiation-induced, immunologically mediated regression of an established tumor as an example of successful therapeutic immunomanipulation. Preferential elimination of suppressor T cells allows sustained production of effector T cells, J. Exp. Med. 164 (1986) 1652–1666.
[20] B. Zhang, N.A. Bowerman, J.K. Salama, H. Schmidt, M.T. Spiotto, A. Schietinger, P. Yu, Y.X. Fu, R.R. Weichselbaum, D.A. Rowley, et al., Induced sensitization of tumor stroma leads to eradication of established cancer by T cells, J. Exp. Med. 204 (2007) 49–55.
[21] T. Tanimoto, A. Hori, M. Kami, Sipuleucel-T immunotherapy for castration-resistant prostate cancer, N. Engl. J. Med. 363 (2010) 1966 author reply 1967–1968.
[22] S. Walter, T. Weinschenk, A. Stenzl, R. Zdrojowy, A. Pluzanska, C. Szczylik, M. Staehler, W. Brugger, P.Y. Dietrich, R. Mendrzyk, et al., Multipeptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival, Nat. Med. 18 (2012) 1254–1261.
[23] D.T. Le, A. Wang-Gillam, V. Picozzi, T.F. Greten, T. Crocenzi, G. Springett, M. Morse, H. Zeh, D. Cohen, R.L. Fine, et al., Safety and survival with GVAX pancreas prime and Listeria Monocytogenes-expressing mesothelin (CRS-207) boost vaccines for metastatic pancreatic cancer, J. Clin. Oncol. 33 (2015) 1325–1333.
[24] H.C. Hoover Jr., J.S. Brandhorst, L.C. Peters, M.G. Surdyke, Y. Takeshita, J. Madariaga, L.R. Muenz, M.G. Hanna Jr., Adjuvant active specific immunotherapy for human colorectal cancer: 6.5-year median follow-up of a phase III prospectively randomized trial, J. Clin. Oncol. 11 (1993) 390–399.
[25] G. Giaccone, C. Debruyne, E. Felip, P.B. Chapman, S.C. Grant, M. Millward, L. Thiberville, G. D'Addario, C. Coens, L.S. Rome, et al., Phase III study of adjuvant vaccination with Bec2/bacille Calmette-Guerin in responding patients with limited-disease small-cell lung cancer (European Organisation for Research and Treatment of Cancer 08971-08971B; Silva Study), J. Clin. Oncol. 23 (2005) 6854–6864.
[26] S. Vassilaros, A. Tsibanis, A. Tsikkinis, G.A. Pietersz, I.F. McKenzie, V. Apostolopoulos, Up to 15-year clinical follow-up of a pilot Phase III immunotherapy study in stage II breast cancer patients using oxidized mannan-MUC1, Immunotherapy 5 (2013) 1177–1182.
[27] A.M. Eggermont, S. Suciu, P. Rutkowski, J. Marsden, M. Santinami, P. Corrie, S. Aamdal, P.A. Ascierto, P.M. Patel, W.H. Kruit, et al., Adjuvant ganglioside GM2-KLH/QS-21 vaccination versus observation after resection of primary tumor > 1.5 mm in patients with stage II melanoma: results of the EORTC 18961 randomized phase III trial, J. Clin. Oncol. 31 (2013) 3831–3837.
[28] H.J. Park, R.J. Griffin, S. Hui, S.H. Levitt, C.W. Song, Radiation-induced vascular damage in tumors: implications of vascular damage in ablative hypofractionated radiotherapy (SBRT and SRS), Radiat. Res. 177 (2012) 311–327.
[29] M. Garcia-Barros, F. Paris, C. Cordon-Cardo, D. Lyden, S. Rafii, A. Haimovitz-Friedman, Z. Fuks, R. Kolesnick, Tumor response to radiotherapy regulated by endothelial cell apoptosis, Science 300 (2003) 1155–1159.
[30] Y. Lee, S.L. Auh, Y. Wang, B. Burnette, Y. Wang, Y. Meng, M. Beckett, R. Sharma, R. Chin, T. Tu, et al., Therapeutic effects of ablative radiation on local tumor require CD8+ T cells: changing strategies for cancer treatment, Blood 114 (2009) 589–595.
[31] A. Gupta, H.C. Probst, V. Vuong, A. Landshammer, S. Muth, H. Yagita, R. Schwendener, M. Pruschy, A. Knuth, M. van den Broek, Radiotherapy promotes tumor-specific effector CD8+ T cells via dendritic cell activation, J. Immunol. 189 (2012) 558–566.
[32] K.D. Moynihan, C.F. Opel, G.L. Szeto, A. Tzeng, E.F. Zhu, J.M. Engreitz, R.T. Williams, K. Rakhra, M.H. Zhang, A.M. Rothschilds, et al., Eradication of large established tumors in mice by combination immunotherapy that engages innate and adaptive immune responses, Nat. Med. 22 (2016) 1402–1410.
[33] C. Vanpouille-Box, A. Alard, M.J. Aryankalayil, Y. Sarfraz, J.M. Diamond, R.J. Schneider, G. Inghirami, C.N. Coleman, S.C. Formenti, S. Demaria, DNA exonuclease Trex1 regulates radiotherapy-induced tumor immunogenicity, Nat. Commun. 8 (2017) 15,618.