A Photodynamically Sensitized Dendritic Cell Vaccine that Promotes the Anti-Tumor effects of Anti-PD-L1 Monoclonal Antibody in Head and Neck Squamous Cell Carcinoma

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Research Article

Keywords: Head and neck squamous cell carcinoma, PD-L1, Immunotherapy, Immune checkpoint, DC vaccine, Photodynamic therapy

Posted Date: July 20th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1718268/v1

Abstract

Background. Immune checkpoint inhibitors are promising tools in combating several cancers, including head and neck squamous cell carcinoma (HNSCC). However, a substantial portion of HNSCC patients do not respond to PD-L1 antibody. Here we describe a photodynamic therapeutic (PDT) approach to enhance anti-tumor effects of the anti-PD-L1 antibody.

Methods. Phototoxicity of PDT was confirmed using fluorescence microscopy, Cell Counting Kit-8 (CCK-8), Enzyme Linked Immunosorbent Assay (ELISA) and flow cytometry analyses. Phenotypic and functional maturation of immature DCs (imDCs) induced by PDT were measured using flow cytometry and ELISA. A mouse model was established using the HNSCC line, SCC7, and was used to evaluate therapeutic effects of PDT-DC vaccine in facilitating anti-tumor immunity of PD-L1 antibody.

Results. Immunogenic cell death (ICD) of SCC7 cells was induced by PDT with 0.5 μM of m-THPC and the 5 J/cm^2 of light dose. ICD of SCC7 cells stimulated imDCs maturation. *In vivo* assays suggested that PDT-DC vaccine and anti-PD-L1 mAb synergistically induced anti-tumor immunity and suppressed tumor progression.

Conclusions. PDT-DC vaccine enhances therapeutic effects of PD-L1 antibody, which might provide a novel approach for HNSCC immunotherapy.

Introduction

Head and neck cancer squamous cell carcinoma (HNSCC) rank as the sixth most common cancer worldwide. Approximately 835,000 new cases and 431,000 deaths due to HNSCC were reported globally in 2018(1). Its etiology can trace to major factors like the continuous tobacco exposure, alcohol consumption, betel chewing, and virus infection. Although intensive multimodality managements are performed on HNSCC, little improvement of its five-year survival rate has been noted in the recent decades(2). Due to the unique anatomical structures in the head and neck area, recurrent or metastasis often occur in HNSCC which is the main reason for patient death(3). Thus, there is a greater need to find new therapies for HNSCC treatment.

Recently, immunotherapy has received an unprecedented attention due to the success of immune checkpoint inhibitors, such as anti-CTLA4(4), anti-PD-1(5) and anti-PD-L1(6) which have been confirmed in multiple clinical trials, including recurrent and metastatic HNSCC(7). However, these immune checkpoint inhibitors are only beneficial to a subset of patients because of dysfunctions of $CD8^+$ cytotoxic T-lymphocytes (CTL)(8). Moreover, several studies indicated that HNSCC cells are unable to be recognized by CTL *in vitro*(9).

Dendritic cells (DCs) as the most professional antigen-presenting cells have a unique role in activating CTLs against tumor. To this end, various DC vaccines have been used in animal models(10, 11). Currently, a number of adjuvants (such as chemokines(12), tumor antigen fusion protein(13), and tumor antigen

peptide(14)) have been tested for promoting the efficacy of DCs, which have made great advancements in DC vaccines. However, a majority of them have not met with the required safety and efficacy levels for clinical use(15). Thus, a search for new methods to facilize antigen-presenting function of DCs remains of great significance.

Photodynamic therapy (PDT) is an emerging minimally invasive approach, which has been approved by US Food and Drug Administration for the treatment of some cancers and benign diseases(16). It is based on interaction between photosensitizers and light of a specific wavelength, this procedure contributes to reactive oxygen species (ROS) generation and finally cell death. Recently, it has been suggested that PDT induces a specific type of immunogenic cell death (ICD) in several cancer cells(17–19). ICD manifests as release of damage-associated molecular patterns (DAMPs), and mitochondrial dysfunction and endoplasmic reticulum stress induced by ROS(20) which leads to further antigenic spread(21) and DC recruitment(22). In high grade glioma, Grag et al. have found that PDT was an efficient method to develop DC vaccines since it pulsed DC maturation and functional activation(23). Thus, PDT strongly activates antigen-presenting DCs.

HNSCC is considered as an immunosuppressive disease because it can evade immune surveillance via downregulating immunogenicity and activating immunosuppressive pathways(24). Hence, new approaches are still needed for improving immune responses to HNSCC. These findings inspired us to develop a strategy involving ICD induced by PDT, which was able to induce maturation and activation of DCs efficiently. We also aimed to demonstrate PDT-DC vaccine in promoting PD-L1 monoclonal antibody (mAb) therapeutic efficacy in a mice model of squamous cell carcinoma.

Materials And Methods

Cell lines and cell culture

The SCC7 cells were obtained from FuHeng Biology (Shanghai, China). SCC7 cells were maintained in RPMI-1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS; FuHeng Biology, Shanghai, China), 100 units/mL of penicillin (HyClone, South Logan, UT), and 100 μg/mL streptomycin (HyClone) at 37 ℃ and 5% CO_2. The authenticity of cell lines has been ensured using STTR markers, before using in experiments.

Cellular uptake of m-THPC

SCC7 cells were cultured in black 96-well microplates with a clear glass bottom (Corning, USA) at a density of 6,000 cells/well. After reaching a confluence of 60–70%, the cells were washed twice with PBS and then incubated with 5,10,15,20-Tetrakis (3-hydroxyphenyl) chlorin (m-THPC or, Temoporfin) (MedChem Express, NJ, USA) of different concentrations (ranging from 0.5 to 10 μM) in serum-free medium in the dark for 24 h. SCC7 cells without treatment were used as a negative control. Intake of m-THPC by the SCC7 cells was detected using an inverted fluorescence emission microscope (IX71, Olympus, Japan) and quantified using a microplate reader (FLUOstar Omega, BMG LABTECH GmbH,

Ortenberg, Germany). The fluorescence excitation was set at 460 nm and the emission was monitored at 650 nm. The fluorescence intensity was corrected for autofluorescence background (n = 5). CCK-8 (Sangon Biotech, Shanghai, China) was performed according to the manufacturer's instructions and the optical density was measured at 450 nm.

Photodynamic treatment-induced ICD

SCC7 cells were incubated in the dark with m-THPC at indicated concentration for 24 h, then irradiated using a laser beam (Changchun Institute of Optics, Fine Mechanics & Physics, Chinese Academy of Sciences, Changchun, China) at 635 nm with different light doses (0.1 to 5 J/cm^2) in photosensitizer-free media, SCC7 cells without irradiation were used as a negative control. Cell death was analyzed by CCK-8 assay after 24 h.

ICD assays

For detecting ICD, SCC7 cells (3×10^5/plate) were seeded in 35mm dishes (NEST, Jiangsu, China), and PDT was performed as described above. After 1h of PDT, SCC7 cells suspensions were treated with anti-calreticulin (CRT) antibody (ab196159, 0.5 mg/ml), then incubated for 1 h at room temperature. The assay was run on a BD FACSCalibur flow cytometer. Supernatants were harvested for ATP (Enhanced ATP assay kit, Beyotime Biotechnology, Shanghai, China) and high mobility group protein B1 (HMGB1) quantified by an ELISA kit (Meimian industrial Co., Ltd, Jiangsu, China) after 4 h and 24 h of PDT, respectively. All assays were performed as described by respective manufacturers and measured on a microplate reader (FLUOstar Omega, BMG LABTECH GmbH, Ortenberg, Germany).

Generation of DCs in vitro

Bone marrow was obtained from the femur and tibia of syngeneic C3H/HeN mice. After depleting erythrocytes, cells were seeded (1.5×10^6 cells/mL in 24-well plates) into RPMI-1640 supplemented with 10% FBS, 20 ng/ml granulocyte macrophage colony-stimulating factor (GM-CSF, PeproTech) and 20 ng/ml interleukin-4 (IL-4, PeproTech) 100 U/mL of penicillin, and 100 μg/mL streptomycin. On day 2 and 4, 50% of the spent media were replaced with fresh media containing GM-CSF and IL-4 was added. On day 6, immature DCs (imDCs) were isolated for experiments.

Analysis of DCs in vitro

SCC7 cells were killed using either mTHPC -PDT or three cycles of freeze-thaw lysis. They were then cocultured with imDCs at a 1:1 ratio for 24 h. As a positive control, imDCs were stimulated by 500 ng/ml of E. coli lipopolysaccharide (LPS, Sigma); Maturation of DCs was analyzed using immunofluorescent anti-mouse CD11c PE-Cy7 (N418, Biolegend) and anti-mouse MHC-APC (M5/114.15.2, Biolegend) in accordance with the manufacturer's instructions. Samples were then collected and analyzed using a BD FACSCalibur flow cytometer. After co-incubating imDCs with the m-THPC-PDT-SCC7 cells, the supernatants were harvested and IL-12 and IL-6 were measured using ELISA (Meimian industrial Co., Ltd, Jiangsu, China).

Animal experiments

C3H/HeN mice (female, 6-8weeks old purchased from Vital River Laboratory Animal Technology Co Beijing, China) were challenged subcutaneously on the right flank with 5×10^6 live SCC7 cells. On day 1 (24 h after inoculation), tumor-bearing mice were randomly assigned into four groups (n = 5). On day 3 after tumorigenesis, mice in treatment groups were immunized with PDT-DC vaccines (approximately 1×10^6 PDT-DCs in 0.1 ml PBS were injected subcutaneously around peritumoral regions), or PBS was administered in the control group of mice. To assess PDT-DC vaccine in promoting the therapeutic potency of PD-L1 blockade, mice were administered an additional 100 μg/mice anti-PD-L1 mAb (10F.9G2, BioXcell, West Lebanon, NH) or an equivalent amount of Rat IgG2b isotype control (LTF-2, BioXcell) with the vaccine. Tumor volumes were monitored using a caliper every three days. Mice were sacrificed when the tumors became necrotic or exceeded 2000 mm^3, and serum was obtained for IL-12 and IL-6 measured by ELISA kit (Meimian industrial Co., Ltd, Jiangsu, China). All experiments were performed under an institutionally approved animal protocol.

Immunohistochemistry

Specimens were fixed with 4% paraformaldehyde solution for 24 h at room temperature, then prepared by dehydration, paraffin dipping and embedding, cut into sections, and finally staining with Hematoxylin and Eosin (H&E). Tissue sections were incubated with 2% BSA for 30 minutes at room temperature, and immunohistochemical staining was performed on them with the primary antibodies and then secondary antibodies. The specimens were observed under BX53 fluorescence microscope (Olympus, Japan).

Statistical analysis

All experiments were repeated at least three times. An unpaired t-test was applied for single comparisons between two groups for both *in vitro* and *in vivo* experiments. All data are expressed as the mean ± SD. Statistical analysis was performed using SigmaStat software (SPSS v20; IBM, Armonk, NY, USA). P 0.05 was considered statistically significant.

Results

Cellular uptake of m-THPC in SCC7 cells

SCC7 cells were incubated with different concentrations of m-THPC (0.5 to 10 μM) in a serum-free medium in the dark for 24 h, and analyzed for fluorescence emission first using an inverted fluorescence microscope and then quantified using a microplate reader. The fluorescence intensity increased with m-THPC concentrations (Figs. 1A and 1B). Furthermore, CCK-8 assay was used to evaluate cytotoxicity of m-THPC in SCC7 cells. m-THPC had no effect on cell viability (Fig. 1C); however, a decrease of cell viability was observed when cells were treated at a fixed light dose, which suggested that cell death was induced in SCC7 cells by treatment with PDT (Fig. 1D). Interestingly, m-THPC concentration was not a factor influencing cell viability when treating SCC7 cells with PDT (Fig. 1D). Taken together, 0.5 μM m-THPC was selected as the experimental condition, because it has a similar effect on cell viability to different concentrations under the same light dose.

PDT induces ICD in SCC7 cells

It is known that PDT induces diverse types of cell death(25) depending on the concentration of photosensitizer and the light intensity(26). To explore the optimal light dose of m-THPC-PDT induced ICD, we analyzed the presence of extracellular DAMPs, which induce innate immune response(27–29), a common feature of ICD. In addition, we measured the expression of CRT on the cell surface, and release of ATP and HMGB1 into the extracellular space(20, 30). Immunofluorescent staining with an anti-CRT antibody showed that CRT exposure occurred as early as 1 h after PDT (Fig. 2A and 2B). Notably, CRT on the levels on cell surface increased significantly at the low light dose groups (0.1 and 0.5 J/cm^2) (Fig. 2A and 2B). 1J exposure caused the highest expression of CRT. No further rise in cell surface CRT levels occurred at 2 J/cm^2, and 5 J/cm^2 of light. We also analyzed the release of ATP (Fig. 2C) and HMGB1 (Fig. 2D) induced by PDT. In contrast to the CRT expression, at the low light doses (0.1 and 0.5 J/cm^2) no significant ATP release was noted. A significant rise in level of ATP release was observed at 1J, which continued to increase in light dose dependent manner. For HMGB1, a significant increase in the extracellular medium occurred at the lowest dose of 0.5 J/cm^2 which continued to increase up to 5 J/cm^2. Basing on these observations, we considered that 5 J/cm^2 of (light dose) and 0.5 μM of m-THPC as the best doses for for inducing optimal level of ICD in the SCC7 cells.

PDT-activated ICD of SCC7 induces phenotypic and functional maturation of imDCs

To determine if ICD alters the phenotypic and functional maturation of mouse bone marrow imDCs, we analyzed surface expression of MHC-□ in imDCs and patterns of interleukin-12 (IL-12) and interleukin-6 (IL-6) production, after coculturing with PDT-treated SCC7 cells. We compared imDCs co-cultured with SCC7 cells treated with m-THPC-PDT to those that were exposed to the freeze-thawed (F/T) cell lysates of SCC7 cells (F/T-SCC7). LPS- and sham-treated imDCS were used as positive and negative controls for these studies. As expected, the LPS-treated imDCs had the highest expression of MHC class-II expression on their surface. The m-THPC-PDT treated SCC7 cells caused phenotypic maturation of imDCs, as indicated by the increase of MHC-□ molecules on the cell surface, which was significantly higher than those treated with F/T-SCC7 (Fig. 3B and 3C). Similarly, we also found that imDCs stimulated with m-THPC-PDT displayed a markedly higher level of IL-12 than that stimulated by F/T SCC7 cells (Fig. 3D). In contrast mTHPC-PDT treated group had a distinctly lower level of IL-6 (Fig. 3E). The LPS-treated cells had significantly higher levels of both IL-6 and IL-12. Collectively, these findings demonstrate that m-THPC-PDT potently induced the phenotypic and functional maturation of imDCs.

PDT-DC vaccine enhances tumor regression induced by anti-PD-L1 mAb in vivo

Treatment with anti-PD-L1 antibody alone imparts significant therapeutic benefits. To explore if PDT-DC vaccine facilitated augments anti-tumor effects of anti-PD-L1 mAb *in vivo*, we used immunocompetent C3H/HeN mice bearing a subcutaneous SCC7 transplant (Fig. 4A). An isotypic control IgG was used as a

negative control, which did not exert any anti-tumor effects. C3H/HeN mice immunized with PDT-DCs or anti-PD-L1 mAb alone significantly reduced tumor growth compared to the IgG control. However, the mice that received the combination treatment had the lowest tumor burden (Fig. 4B). The combination treatment strongly reduced tumor volume and tumor weight compared to either drug alone (Fig. 4C and 4D). Histopathological analyses did not reveal any significant damage to the heart, lung, spleen, kidney and liver following the various treatments used in this study (Fig. 4E). The body weights of mice treated with the combination was not significantly different from the controls indicating the absence of any side effects (Fig. 4F). IHC analysis was used to confirm the changes in expression of growth-related markers. As shown in Fig. 4G, cell proliferation index Ki-67 shown no differences among these treatments.

PDT-DC vaccine promoting the anti-tumor effects of anti-PD-L1 mAb by activating immune system

The focus of tumor immunotherapy is to restore the killing activity of CTLs. To investigate the reactivation of immune response by the combination treatment, tumors and spleens from treatment groups were tested for the presence of increased numbers of anti-tumor T-cells using specific antibodies. $CD8^+$ and $CD4^+$ T cell numbers in tumors increased dramatically in combination treatment group while shown a decrease of Treg cells ($Foxp3^+$) when compared with other groups in the tumor (Fig. 5A). A similar analysis of spleens showed an increase in $CD8^+$ and $CD4^+$ T cells in tumors, with no significant differences in Treg cells for every group (Fig. 5B). Additionally, secretion of inflammatory factors is crucial for immune system activation. Sera from the mice were also tested for changes in the levels of IL-12 and IL-6. PDT-DC or its combination with anti-PD-L1 antibody significantly increased serum IL-12 levels., compared to the controls. In contrast, IL-6 levels were lower in mice that received the combination therapy. These observations are consistent with those of in vitro studies (Fig. 5C and 5D). Together, our *in vivo* findings confirmed that PDT-DCs were not only an effective tool in activating the immune system but also could be considered as a potent adjuvant therapy for tumors that weakly respond to immune checkpoint therapy.

Discussion

Growing evidence suggests that HNSCC is an immunosuppressive disease, which escapes from immune surveillance through some uncertain mechanisms(31). Classical HLA class I antigen loss/downregulation of HNSCC is associated with tumor immune escape leading to abnormalities of antigen-presentation to CTLs(32). Additionally, HNSCC recruits Treg cells, which secrete IL-10 and TGF-β(33, 34) into the tumor microenvironment to inhibit CTLs. The upregulation of PD-L1 on the surface of HNSCC plays a crucial role in inducing apoptosis of tumor infiltrating lymphocytes and hindering differentiation of CTLs, which appears to be a major mechanism for immune escape of HNSCC(35). Therefore, the recent success of immunotherapy of cancer needs additional approaches that further augment and activate antitumor CTLs.

The success of immune checkpoint inhibitors especially anti-PD-L1 mAb has been considered as a promising way for controlling many kinds of tumors and highlights the specific role of T cells in immunotherapy. Unfortunately, many clinical trials confirmed that high level of PD-L1 expression on the surface of cancer cells does not mean a high response rate to anti-PD-L1 mAb(36, 37). Moreover, longterm use of anti-PD-L1 mAb also has an undesirable side effect such as an autoimmune-like damage of normal tissues because of a global non-specific activation of other CTLs. Thus, a therapy that activating CTLs to selectively kill tumor cells is necessary at this time.

PDT is a potential approach for some solid tumors where an interaction between a photosensitizer and a specific wavelength of light leads to immunogenic tumor death(38). This process contributes to a generation of intracellular ROS (in the tumor) which cause cytotoxicity. Accumulating evidence supports the notion that cancer cells undergo ICD, which potently stimulates the antigen presenting cells (APCs) unlike other types of cell deaths, and is associated with the release of DAMPs into the tumor microenvironment(20). Notably, ICD uniquely stimulates DC maturation because it provides specific tumor associated antigens.

We considered that the PDT sensitized DC vaccine may present specific HNSCC associated antigens to CTLs which were activated by anti-PD-L1 mAb. Likewise, this kind of DC vaccine is able to overcome limitations of application of anti-PD-L1 mAb. Indeed, the combination of m-THPC and a light dose were able cause the release of DAMPs and potently stimulated imDCs to differentiate (Figs. 2 and 3). Currently, a number of adjuvants (such as chemokines(12), tumor antigen fusion protein(13), and tumor antigen peptide(14)) have been proved good stimulations for DC vaccines preparation. Freeze-thawed tumor cells were the most common used method to obtain those adjuvants. We compared stimulating effects of freeze-thawed tumor cells and PDT-induced ICD on DCs maturation intentionally. As shown in Fig. 3, in terms of stimulating morphological and functional maturation of DCs, PDT-induced ICD was far superior to that of freeze-thawed tumor cells. DCs stimulated by PDT can recognize antigens of squamous cell carcinoma and present these antigens to CTLs. In vivo, the combination treatment not only reduced the tumor proliferation stronger than the controls (Fig. 4) but also increased a high level of $CD8^+$ and $CD4^+$ T cells infiltration. Simultaneously, we also noted a decrease of immunosuppressive Tregs in the tumor. In addition, the combination treatment also affected the anti-tumor immune response in other organs, as observed by a rise in the $CD8^+$ and $CD4^+$ T cells in the spleen, increasing the secretion of pro-inflammatory cytokine IL-12, and decreasing the secretion of pro-tumor inflammatory factor IL-6 (Fig. 5). It is also noteworthy that the combination treatment has no side effects such as a generalized damage of normal tissues on in the tumor bearing mice (Fig. 4).

In summary, we demonstrated that PDT sensitized DCs can robustly promote the anti-tumor effects of anti-PD-L1 mAb. Moreover, the current study reveals the application of a DC vaccine as an adjuvant since it was commonly used as the single method in immunotherapy.

Abbreviations

HNSCC: head and neck squamous cell carcinoma; CTL: cytotoxic T-lymphocytes; DC: dendritic cell; PDT: photodynamic therapy; ROS: reactive oxygen species; ICD: immunogenic cell death; DAMPs: damage-associated molecular patterns; mAb: monoclonal antibody; CRT: calreticulin; HMGB1: high mobility group protein B1; F/T: freeze-thawed; LPS: *E. coli* lipopolysaccharide; Interleukin-12: IL-12; Interleukin-6: IL-6.

Declarations

Ethics approval and consent to participate

All animal experiments were performed in accordance with institutional guidelines and approved by the ethics committee of Basic Medical Sciences of Jilin University approved the study.

Consent for publication

All authors consent for publication.

Competing interests

The authors have no conflict of interest to declare that there are no conflicts of interest.

Funding

The current study was funded by the National Natural Science Foundation of China (grant numbers 81773217 to L. Zhang), Research Fund of Jilin Provincial Science and Technology Department (grant numbers 20190701065GH and 20200404120YY to L. Zhang), Jilin Province Health Technology Innovation Project (grant numbers 2019J030 to L. Zhang), the Medical and Health Personnel Special Project of Jilin Province (Grant numbers 2019SCZT012 to D. Yu), the Natural Science Foundation of Jilin Province (Grant numbers 20190201212JC to D. Yu), International Cooperation Project from Science and Technology Department of Jilin Province (Grant numbers 20180414054GH to D. Yu). Graduate Innovation Fund of Jilin University (grant number 101832020CX291 to S. Li). D.V. Kalvakolanu is supported by the Cigarette Restitution Funds of the University of Maryland Greenebaum NCI Comprehensive Cancer Center.

Authors' contributions

DY and LZ designed the experiment. SL conducted the experiment and wrote this manuscript. DW, JZC, XZ, DW and JCS analyzed and collected the data. DK provided critical comments, concepts, and insights. All authors contributed to the article and approved the submitted version.

Availability of data and materials

All data that support the findings of this study are available from the corresponding authors upon reasonable request.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Cancer J Clin. 2018;68(6):394–424.
2. Obid R, Redlich M, Tomeh C. The Treatment of Laryngeal Cancer. Oral Maxillofac Surg Clin N Am. 2019;31(1):1–11.
3. Zhang X, Zhang L, Tan X, Lin Y, Han X, Wang H, et al. Systematic analysis of genes involved in oral cancer metastasis to lymph nodes. Cell Mol Biol Lett. 2018;23:53.
4. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711–23.
5. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366(26):2443–54.
6. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366(26):2455–65.
7. Stafford M, Kaczmar J. The neoadjuvant paradigm reinvigorated: a review of pre-surgical immunotherapy in HNSCC. Cancers of the head & neck. 2020;5:4.
8. Matsuo K, Yoshie O, Kitahata K, Kamei M, Hara Y, Nakayama T. Recent Progress in Dendritic Cell-Based Cancer Immunotherapy. Cancers. 2021;13(10).
9. López-Albaitero A, Nayak JV, Ogino T, Machandia A, Gooding W, DeLeo AB, et al. Role of antigen-processing machinery in the in vitro resistance of squamous cell carcinoma of the head and neck cells to recognition by CTL. Journal of immunology (Baltimore, Md: 1950). 2006;176(6):3402-9.
10. Oba T, Long MD, Keler T, Marsh HC, Minderman H, Abrams SI, et al. Overcoming primary and acquired resistance to anti-PD-L1 therapy by induction and activation of tumor-residing cDC1s. Nat Commun. 2020;11(1):5415.
11. Zhu S, Lv X, Zhang X, Li T, Zang G, Yang N, et al. An effective dendritic cell-based vaccine containing glioma stem-like cell lysate and CpG adjuvant for an orthotopic mouse model of glioma. Int J Cancer. 2019;144(11):2867–79.
12. Biragyn A, Surenhu M, Yang D, Ruffini PA, Haines BA, Klyushnenkova E, et al. Mediators of innate immunity that target immature, but not mature, dendritic cells induce antitumor immunity when genetically fused with nonimmunogenic tumor antigens. Journal of immunology (Baltimore, Md: 1950). 2001;167(11):6644-53.
13. Mittal D, Vijayan D, Putz EM, Aguilera AR, Markey KA, Straube J, et al. Interleukin-12 from CD103(+) Batf3-Dependent Dendritic Cells Required for NK-Cell Suppression of Metastasis. Cancer Immunol Res. 2017;5(12):1098–108.
14. Alexandre YO, Ghilas S, Sanchez C, Le Bon A, Crozat K, Dalod M. XCR1 + dendritic cells promote memory CD8 + T cell recall upon secondary infections with Listeria monocytogenes or certain viruses. J Exp Med. 2016;213(1):75–92.

15. Wculek SK, Cueto FJ, Mujal AM, Melero I, Krummel MF, Sancho D. Dendritic cells in cancer immunology and immunotherapy. Nat Rev Immunol. 2020;20(1):7–24.
16. Triesscheijn M, Baas P, Schellens JH, Stewart FA. Photodynamic therapy in oncology. Oncologist. 2006;11(9):1034–44.
17. Garg AD, Krysko DV, Vandenabeele P, Agostinis P. The emergence of phox-ER stress induced immunogenic apoptosis. Oncoimmunology. 2012;1(5):786–8.
18. Panzarini E, Inguscio V, Fimia GM, Dini L. Rose Bengal acetate photodynamic therapy (RBAc-PDT) induces exposure and release of Damage-Associated Molecular Patterns (DAMPs) in human HeLa cells. PLoS ONE. 2014;9(8):e105778.
19. Tanaka M, Kataoka H, Yano S, Sawada T, Akashi H, Inoue M, et al. Immunogenic cell death due to a new photodynamic therapy (PDT) with glycoconjugated chlorin (G-chlorin). Oncotarget. 2016;7(30):47242–51.
20. Garg AD, Nowis D, Golab J, Vandenabeele P, Krysko DV, Agostinis P. Immunogenic cell death, DAMPs and anticancer therapeutics: an emerging amalgamation. Biochim Biophys Acta. 2010;1805(1):53–71.
21. He C, Duan X, Guo N, Chan C, Poon C, Weichselbaum RR, et al. Core-shell nanoscale coordination polymers combine chemotherapy and photodynamic therapy to potentiate checkpoint blockade cancer immunotherapy. Nat Commun. 2016;7:12499.
22. Byun DJ, Wolchok JD, Rosenberg LM, Girotra M. Cancer immunotherapy - immune checkpoint blockade and associated endocrinopathies. Nat reviews Endocrinol. 2017;13(4):195–207.
23. Garg AD, Vandenberk L, Koks C, Verschuere T, Boon L, Van Gool SW, et al. Dendritic cell vaccines based on immunogenic cell death elicit danger signals and T cell-driven rejection of high-grade glioma. Sci Transl Med. 2016;8(328):328ra27.
24. Elmusrati A, Wang J, Wang CY. Tumor microenvironment and immune evasion in head and neck squamous cell carcinoma. Int J Oral Sci. 2021;13(1):24.
25. Acedo P, Stockert JC, Cañete M, Villanueva A. Two combined photosensitizers: a goal for more effective photodynamic therapy of cancer. Cell Death Dis. 2014;5(3):e1122.
26. Turubanova VD, Balalaeva IV, Mishchenko TA, Catanzaro E, Alzeibak R, Peskova NN, et al. Immunogenic cell death induced by a new photodynamic therapy based on photosens and photodithazine. J Immunother Cancer. 2019;7(1):350.
27. Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med. 2007;13(9):1050–9.
28. Yamazaki T, Hannani D, Poirier-Colame V, Ladoire S, Locher C, Sistigu A, et al. Defective immunogenic cell death of HMGB1-deficient tumors: compensatory therapy with TLR4 agonists. Cell Death Differ. 2014;21(1):69–78.
29. Krysko O, Løve Aaes T, Bachert C, Vandenabeele P, Krysko DV. Many faces of DAMPs in cancer therapy. Cell Death Dis. 2013;4(5):e631.

30. Garg AD, Krysko DV, Vandenabeele P, Agostinis P. DAMPs and PDT-mediated photo-oxidative stress: exploring the unknown. Photochemical & photobiological sciences: Official journal of the European Photochemistry Association and the European Society for Photobiology. 2011;10(5):670–80.
31. Lalami Y, Awada A. Innovative perspectives of immunotherapy in head and neck cancer. From relevant scientific rationale to effective clinical practice. Cancer Treat Rev. 2016;43:113–23.
32. Campoli M, Chang CC, Oldford SA, Edgecombe AD, Drover S, Ferrone S. HLA antigen changes in malignant tumors of mammary epithelial origin: molecular mechanisms and clinical implications. Breast Dis. 2004;20:105–25.
33. Kerkar SP, Restifo NP. Cellular constituents of immune escape within the tumor microenvironment. Cancer Res. 2012;72(13):3125–30.
34. Mempel TR, Pittet MJ, Khazaie K, Weninger W, Weissleder R, von Boehmer H, et al. Regulatory T cells reversibly suppress cytotoxic T cell function independent of effector differentiation. Immunity. 2006;25(1):129–41.
35. Zheng A, Li F, Chen F, Zuo J, Wang L, Wang Y, et al. PD–L1 promotes head and neck squamous cell carcinoma cell growth through mTOR signaling. Oncol Rep. 2019;41(5):2833–43.
36. Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014;515(7528):563–7.
37. Powles T, Eder JP, Fine GD, Braiteh FS, Loriot Y, Cruz C, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. Nature. 2014;515(7528):558–62.
38. Vrouenraets MB, Visser GW, Snow GB, van Dongen GA. Basic principles, applications in oncology and improved selectivity of photodynamic therapy. Anticancer Res. 2003;23(1b):505–22.

Figures

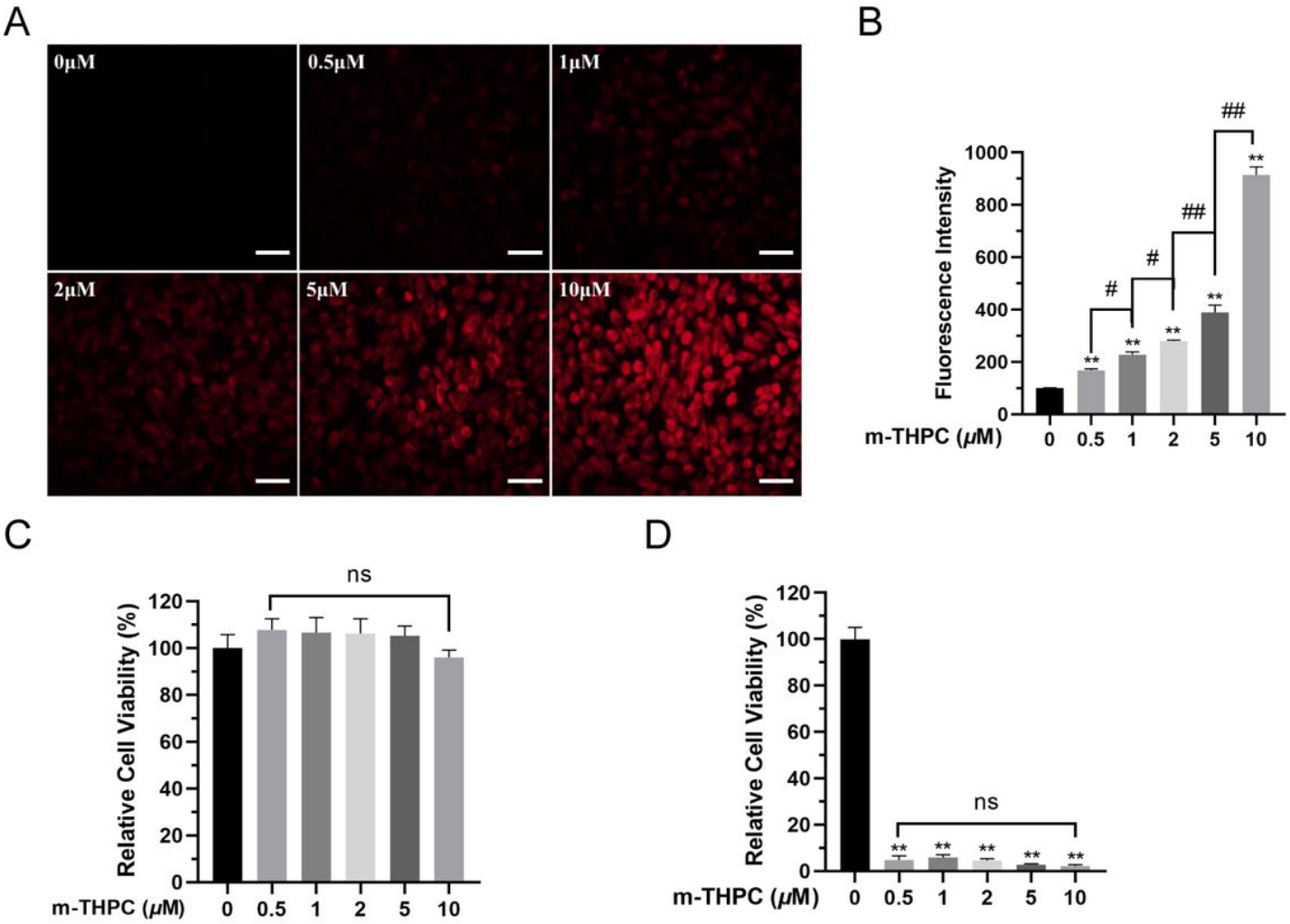

Figure 1

Accumulation of m-THPC in SCC7 cells after incubation for 24 h. (A) Detection by an inverted fluorescence microscope, the increased red fluorescence was observed with the increased amount of m-THPC (0.5 to 10 μM) incubated in SCC7 cells (Scale Bars=20 μm). (B) FI was measured at 650 nm using a microplate reader, high FI was observed when increasing the dose of m-THPC. (C) SCC7 cells were only treated with m-THPC (0.5 to 10 μM) for 24 h, cell viability was determined by CCK-8 assay. (D) After incubation with m-THPC (0.5 to 10 μM), SCC7 cells were treated with a fixed light dose (1 J/cm²), and cell viability was tested by CCK-8 assay. The results were obtained from three independent experiments and expressed as the mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ among the groups. FI, fluorescence intensity.

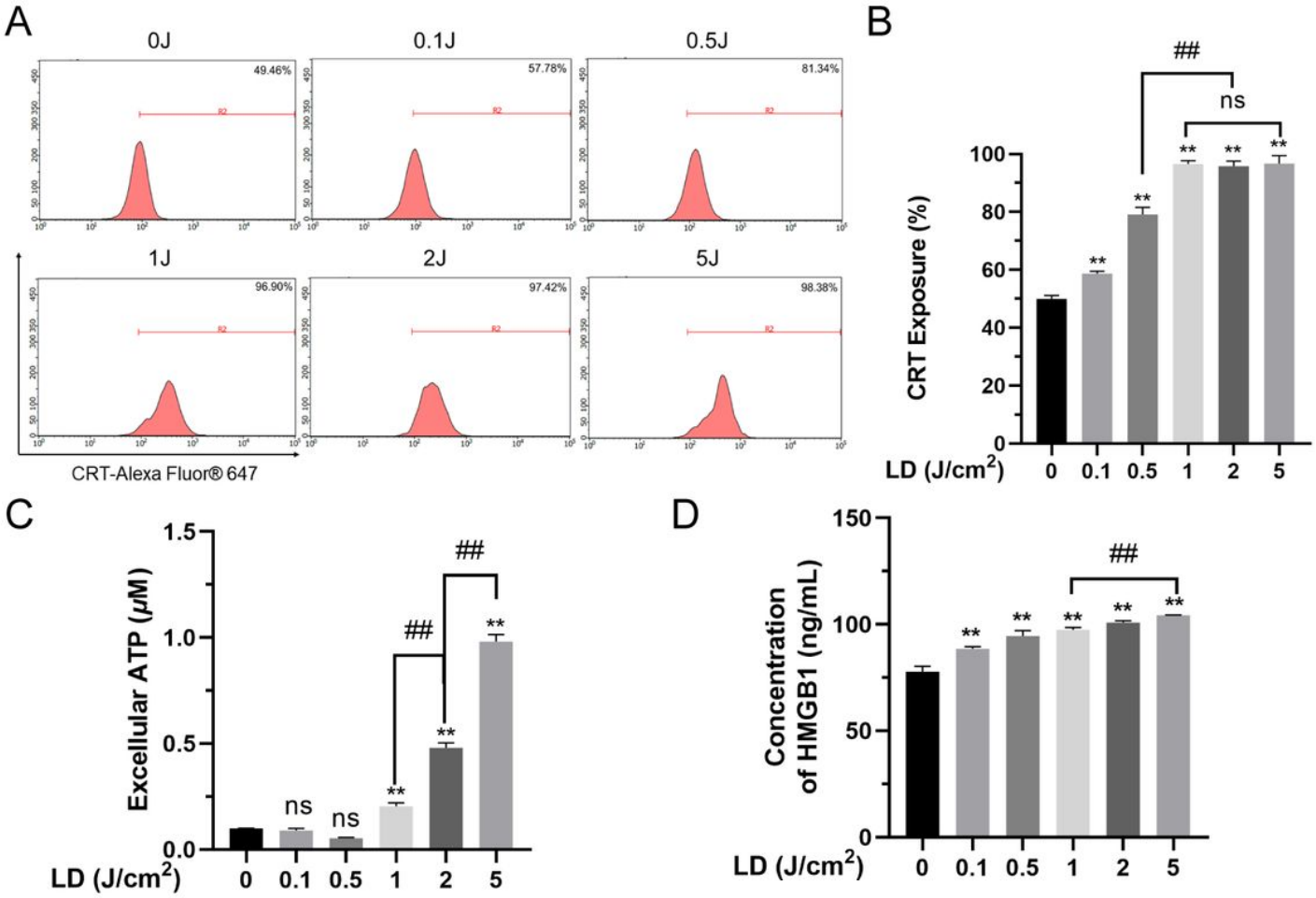

Figure 2

ICD in SCC7 cells induced by m-THPC-PDT is associated with DAMPs. (A) Quantification of flow cytometry analysis of CRT exposure at the cell surface. SCC7 cells were recovered 1 h after the treatment with PDT at different LD (0.1 to 5 J/cm²) (n=3). (B) SCC7 cells were recovered for 4 h after PDT treatment and ATP was measured in the supernatants. ATP values represent obvious increase relative to untreated cells and low LD groups (n=3). (C) SCC7 cells were recovered for 24 h after PDT treatment and HMGB1 was measured in the supernatants by ELISA (n=3). The results were obtained from three independent experiments and expressed as the mean ± SD. ** $P < 0.01$ vs. control, $^{\#\#}$ $P < 0.01$ among the groups. LD: light dose.

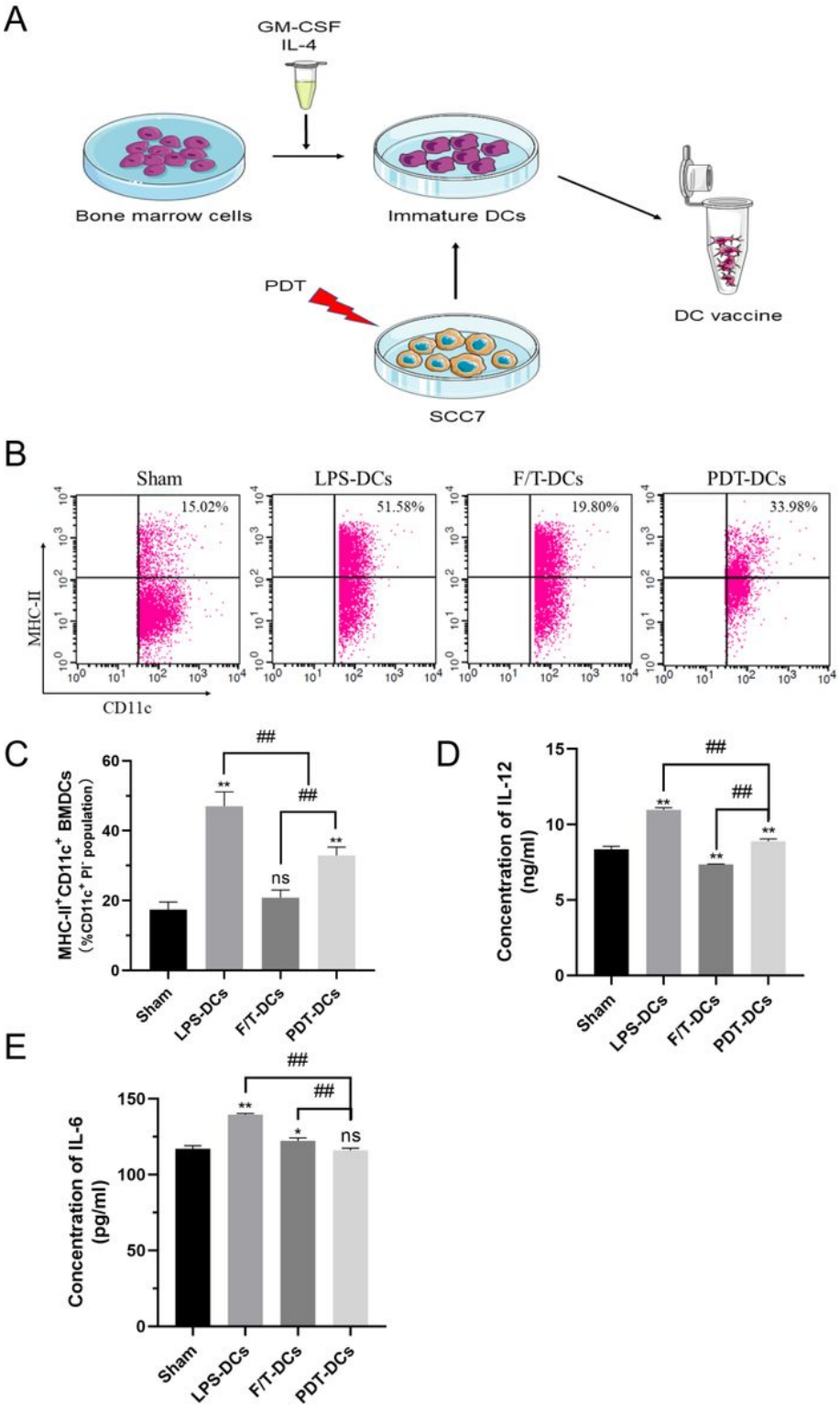

Figure 3

Analysis of imDCs maturation in vitro. (A) Generation of PDT-stimulated DCs. (B) and (C) Phenotypic maturation into DCs. imDCs were exposed for 24 h to SCC7 cells that were either pre-treated with m-THPC-PDT treatment or three cycles of F/T SCC7 cells, followed by the detection of MHC-Ⅱ molecules on the surface of DCs. Cells double positive for Cd11c and MHC-Class II antigens were analyzed. (D) Quantification of IL-12 production in the supernatants of imDCs were measured by ELISA (n=3). The data

from various treatment groups were plotted. (E) This panel is similar to panel D except that the IL-6 production was measured by ELISA (n=3). The results were obtained from three independent experiments and expressed as the mean ± SD. * $P < 0.05$ vs. control, ** $P < 0.01$ vs. control, $^{\#\#}$ $P < 0.01$ among the groups. DCs: dendritic cells; imDCs: immature DCs; F/T DCs: freeze and thaw;

Figure 4

PDT-DC vaccine enhances tumor regression induced by anti-PD-L1 mAb in vivo. (A) Schematic representation of treatment protocol for HNSCC model. (B) Images of the resected tumors are shown. (C) Tumor growth in mice after 14-days various treatments. (D) Tumor weights after 14-days of various treatments. (E) H&E staining of the heart, liver, spleen, lung, and kidney, 14 days after treating with the indicated agents (scale bars = 25 *μm*). (F) The body weights of mice were measured every three days for each group. (G) IHC analyses of tumor tissues for the expression of Ki67 (scale bars= 25 *μm*).

Figure 5

PDT-DC vaccine promoting the anti-tumor effect of anti-PD-L1 mAb by activating immune system. (A) IHC analyses of tumor tissues for the presence of $CD8^{+}$, $CD4^{+}$and $Foxp3^{+}$ cells (scale bars= 20 *μm*). (B) and (D) Serum levels of IL-12 and IL-6 in mice treated with the indicated agents. Cytokines were measured using ELISA (n=3). (C) IHC analyses of spleen sections for the $CD8^{+}$, $CD4^{+}$and $Foxp3^{+}$ cells after treating the mice with the indicated agents (scale bars= 20 *μm*). The results of serum levels of IL-12 and IL-6 were obtained from three independent experiments and expressed as the mean ± SD. * $P < 0.05$ vs. control, ** $P < 0.01$ vs. control, $^{\#\#}$ $P < 0.01$ among the groups.

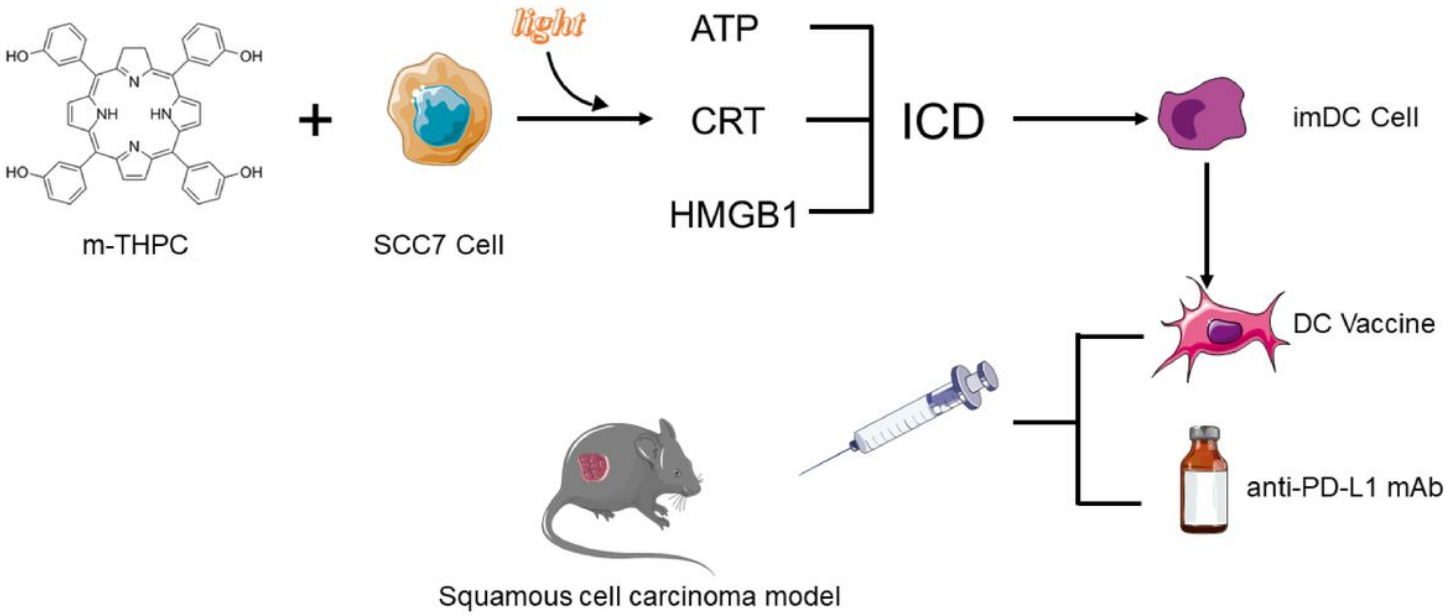

Figure 6

Graphic abstract.