

Enhancing Cancer Immunotherapy: The Role of Nanoparticle-Mediated Immunogenic Cell Death

Karobi Dey¹, Bidisha Ghosh¹, Subhasis Sarkar²

¹Department of Biotechnology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata-700121

²Department of Microbiology, School of Life Sciences, Swami Vivekananda University, Barrackpore, Kolkata-700121.

*Corresponding Author's Email: subhasiss@svu.ac.in

Abstract

Cancer immunotherapy has caused a revolution in cancer treatment, but it faces a big problem: many tumor cells don't set off a strong immune response. When cancer cells die during immunogenic cell death (ICD), they need to send out danger signals to kick-start anti-tumor immune responses. Our study looks at using nanoparticles as a new way to trigger ICD and make cancer immunotherapy work better. We show that our nanoparticles can target and kill cancer cells on purpose, which makes them release ATP HMGB1, and calreticulin. These danger signals help to call in immune cells, turn on dendritic cells, and get them to make pro-inflammatory cytokines, which leads to T cells that can spot tumors. What's more, this ICD method using nanoparticles works well with checkpoint blockade and cancer vaccines making them better at fighting tumors in early tests. This strategy may have the potential to break the barriers currently associated with cancer immunotherapy and provide the ability to design more effective and personalized treatments for cancer patients.

Keyword: Cancer, Immunogenic cell death, Immunotherapy, Nanoparticles.

INTRODUCTION

The establishment of a strong anticancer immune response is possible through the interaction between immune cells and antigens released by dying cancer cells. The mode of cell death can impact the quality and quantity of tumor immunogenicity and may require the tumor cells to undergo immunogenic cell death. Inducers of immunogenic cell death are commonly used in clinics to kill tumor cells by either resembling or directly exposing danger signals, thereby stimulating the immune system to respond to cancer with increased efficiency. However, the problems with currently developed immunogenic cell death-inducing drugs are that most of them have severe side effects, and the timing of the drug administration in relation to the immune modulator coupling is unclear. (Chen DS, 2013)

Within this perspective, using nanomaterials to enhance immunogenic cell death efficacy may represent a solution for important barriers faced by conventional inducers. Numerous types of nanoparticles have demonstrated the capability of inducing immunogenic cell death, thereby suggesting that the classical small-molecule inducers could be partially replaced by nanotechnologies. Moreover, nanoparticle-enabled immunogenic cell death may enhance the efficacy of cancer immunotherapy through both a direct effect on the immune system and the combination with adjuvant-based tumor vaccination(Ribas A, 2018). Here, we focus on several emerging classes of nanoparticle- assisted immunogenic cell death inducers, some of which have potential combined photothermal/immunotherapy properties, and illustrate the involvement of immunogenic cell death in successful anticancer immunotherapy synergistically coupled with physical treatment. We also discuss the remaining issues related to the broad applications of nanoparticle-assisted immunogenic cell death inducers in the field of cancer immunotherapy and current strategies to overcome them. (Dranoff G, 2004)

Immunogenic Cell Death (In Situ Cancer Vaccination)

In ICD, it releases DAMPs and TAAs, which then are internalized by DCs and macrophages and consequently processed and displayed to adaptive immunity cells for mounting an antigen-specific immune response. Several DAMPs are also known, involving apoptotic cell death; such as calreticulin exposed at the pre-apoptotic surface of the cell, release of ATP in the blebbing phase of apoptosis, and finally release of post-apoptotic high mobility group box 1 and heat shock proteins. ATP is a chemoattractant that recruits APCs, while CRT serves as an “eat-me” signal to enhance the uptake of dying tumor cells and their debris by APCs. (Restifo NP, 2012) Furthermore, HMGB-1 and HSPs augment optimal antigen presentation to T cells. ICD can also trigger a strong inflammatory response by releasing pro-inflammatory cytokines such as TNF- α , IL-6, and IL-1 β . These approaches are simple, and ethically good, and effective in inducing selective and strong immunity through local in situ cell death and systemic cell-mediated immune responses. Utilization of ICD effects of conventional treatment methods will enhance the infiltration of immune effector cells transforming an immunosuppressive TME into an immunogenic TME, thereby increasing patient response rates to immune adjuvants and checkpoint blockade immunotherapy. (Restifo NP, 2012)

Design of Nanoparticles for ICD

In situ cancer vaccines require the ICD inducer to precisely focus on tumors. In contrast to traditional methods, nanotechnology provides a means to deliver an optimal dose of ICD inducers to targeted tissues or cell types efficiently, thereby boosting their effectiveness while minimizing adverse effects. NPs (nanoparticles) are capable of co-loading multiple components for concurrent delivery, shielding the payloads from degradation and early release, and targeting tumors either passively or actively through the enhanced permeability and retention (EPR) effect or by surface modification with ligands. (Gubin MM, 2014) Generally, polymeric NPs are tailored with specific shapes and sizes for the intracellular release of loaded small molecules, as well as surface functionalization to surpass biological barriers.

Typically, polymeric NPs are designed with selective shapes and sizes, for intracellular release of loaded small molecules, and surface functionalization for delivery beyond biological barriers. Furthermore, inorganic NPs can be used as a localized source of ICD-inducing treatment or accentuate treatment by external energy fields to minimize damage to healthy tissues. Metals in inorganic NPs are chosen for biocompatibility and intrinsic behavior such as magnetic susceptibility and colloidal stability. NPs that possess imaging capabilities can track the location and therapeutic outcomes in real-time, while NPs with immunomodulatory effects can serve as adjuvants or immune enhancers. In addition, NPs can be specifically designed regarding size, shape, structure, payload, and surface characteristics to facilitate their transportation and that of their cargoes across biological barriers while improving accumulation in solid tumors.(H, 2015)

Application of Nanoparticles in Enhancing Cancer Immunotherapy

1. Hyperthermia

Hyperthermia (HT), the heating of tissue to 39–45°C, was shown to activate an antitumor immune response by inducing a cell stress known as the unfolded protein response, which leads to up-regulation of HSPs on the cell membrane and TAAs within the tumor cells. The released TAAs and TAA-HSP complexes aid in uptake by APCs and assist in antigen presentation via MHC class I, activating antigen-specific killer T cells that undergo clonal expansion and migrate to all tumors (both primary and metastatic) to directly eliminate tumor cells. Additionally, the fever-like temperature induced by HT is shown to enhance antigen presentation, boost tumor blood flow, and speed up leukocyte movement to the tumor.(Evans SS, 2015) Both the effectiveness of treatment and the resulting immune response were found to depend on the thermal dose, necessitating careful optimization to balance the treatment of primary tumors and the induction of immune effects against distant tumors. For instance, coagulative ablation techniques, where high temperatures effectively “liquefy” the tumor tissue and obstruct intertumoral (i.t.) blood circulation, demonstrated significant detrimental impacts on the main

tumor but potentially created a barrier for the infiltration of immune cells and immune activation. Another critical point is that surrounding healthy tissues can also suffer extensive damage during standard hyperthermia treatment, making it a significant technical challenge to selectively heat the tumor area to the targeted temperature while preserving adjacent normal tissues. Luckily, NP-assisted hyperthermia provides a way to regulate the thermal dose and target the treatment locally.(Homma T, 2016)

1.1. Magnetic Liquid Hyperthermia

Magnetic fluid hyperthermia involves the combination of magnetic NPs with an alternating magnetic field (AMF) that induces controlled and uniform local hyperthermia in tumors without any limitation of penetration depth. Toraya-Brown et al. used an external magnetic field to heat tumors up to 43°C for 30 min using 100 nm Fe₃O₄ BNF-Starch magnetic NPs. In another approach, the Kobayashi group has successfully heated local tumor tissue to above 43°C without damaging the surrounding normal tissues in the presence of AMF by the use of cationic liposomes containing MNHT. In both instances, MFH triggered an antitumor immune response mediated by both CD8+ and CD4+ T cells, leading to elimination of both heat-treated primary tumors and unheated distant tumors and subsequent rechallenge rejection. To improve MNHT, magnetic nanoparticles were linked with a melanogenesis substrate, N-propionyl cysteaminyphenol (NPrCAP), allowing for specific uptake by melanoma cells where they react with tyrosinase to generate highly toxic free radicals, leading to chemotherapeutic and immunotherapeutic cell death. Simultaneously, they developed their localized hyperthermia technique alongside immunostimulatory factors; intertumoral injection of IL-2 or GM-CSF 24 hours post-intracellular HT achieved complete tumor regression in 75% and 40% of mice, respectively. The recombinant mouse HSP70 gene and protein also significantly boosted the therapeutic effectiveness of MNHT.(Moy AJ, 2017)(Ito A, 2003)

1.2. Photothermal Therapy

Photothermal therapy is an optical energy-mediated mode of non-invasive hyperthermia which converts the light-based energy to heat for thermal ablation of cancer cells. It is non-toxic in the dark and can be applied locally on the tumor area, leading to a high treatment efficacy with very few side effects. PTT has been known to produce antitumor immunological effects by generating TSAs from the residues of ablated tumor cells. Inorganic nano-agents with inherent capability of NIR light absorption were developed for the delivery of thermal energy and immunoadjuvants. Yata et al. modified AuNPs by conjugation with CpG oligodeoxynucleotides and mixed them with a hexapod-like structured DNA containing CpG sequences, resulting in an immunostimulatory Au-DNA hydrogel. Guo et al. designed chitosan-coated hollow CuS NPs containing CpG, which could break down after laser excitation, reassemble, and transform into polymer complexes, effectively eliminating the primary tumor and simultaneously inhibiting the growth of distant untreated tumors. (Celli JP, 2010)

Small molecule photothermal agents can also be co-loaded with immunostimulators into liposomes or polymeric nanoparticles for effective combination of PTT and immunotherapy. Activation of and increasing infiltration of DCs and CD8⁺ T cells by coating a HA-CpG conjugate onto fluorophore (IR-7)-loaded liposomes led to eradication of tumors in mice and inhibiting metastasis. (Chatterjee DK, 2008)

Radiation Therapy

Radiotherapy (RT) is an effective treatment option for cancer, typically utilized for its capability to destroy cancer cells through the induction of DNA double strand breaks, which is not restricted by the depth of tissue. The surface exposure of CRT and the release of HSP70 and HMGB-1 indicate that RT promotes ICD in situ, enhances the maturation of DCs,

and stimulates IFN γ -producing T cells both in vitro and in vivo. Consequently, combining RT with i.t. administration of the CpG adjuvant has demonstrated preclinical effectiveness and has been evaluated in humans, where it led to the rejection of both the irradiated tumor and tumors outside the radiation area (abscopal effect). Furthermore, RT has been noted to work synergistically with ICB to enhance antitumor immunity. Nevertheless, not all RT-induced changes in the tumor and its microenvironment support immune activation. Recent findings indicate that pro-tumorigenic M2 macrophages are gathering in hypoxic regions of irradiated tumors, alongside a rise in immunosuppressive Treg cells following RT. Interestingly, the dose and fractionation of RT could influence the balance between the growth of effector and Treg cells.(Mason KA, 2012)

Chemotherapeutic Drugs

Though apoptosis has historically been regarded as a non-inflammatory, immunologically silent or even tolerogenic, recent evidence has shown that a subset of chemotherapeutics can be pro-inflammatory and induce ICD. As a systemic agent, chemotherapy has the potential to initiate an immune response in multiple sites. Furthermore, NP-mediated chemotherapy has been reported to enhance ICD and consequently improve antitumor effects of the free ICD inducer. For example, Zhao et al. found that immunogenic oxaliplatin encapsulated in PLGA-mPEG NPs released more DAMPs and induced more dendritic cell and T lymphocyte activation and infiltration than free oxaliplatin, improving anticancer efficacy in immunocompetent mice.(Chen DS, 2013)

Doxorubicin (DOX) is a bonafide ICD inducer that has been widely used in nanoparticle formulations. Zheng et al. developed a pH- and GSH-dual-sensitive delivery system for systemic treatment of highly metastatic triple-negative breast cancer by loading DOX into highly integrated mesoporous silica NPs, which induced DC maturation and antitumor cytokine release. The antitumor efficacy and

immunity induced by DOX can be enhanced by combination with immunotherapy. Su et al. found that pre-treatment with TNF- α pDNA polyplexes 48 h prior to liposomal DOX increased its accumulation in tumors, most likely due to the opening of the tumor endothelial tight junctions by TNF- α . (Zheng D-W, 2016\)

Dual pH-responsive multifunctional NP system for the treatment of breast cancer through the coating of TLR-7/8 agonist (R848)-loaded poly(l-histidine) (PHIS) nanocore with acid-cleavable HA-DOX conjugates. The components were separated in the TME and internalized HA-DOX through CD44-mediated endocytosis by tumor cells. Intracellular HA-DOX released DOX through the hydrolysis of the hydrazone bond at pH $\approx$ 5.5 to induce ICD, whereas extracellular PHIS/R848 released R848 through the ionization of PHIS at pH $\approx$ 6.5 to enhance the immune response.(Su B, 2013)

Another chemotherapeutic with a clinically approved nanoformulation, paclitaxel (PTX), is also known to induce ICD. Roy et al. combined chemo- and immunotherapy using PLGA NPs loaded with PTX and a TLR-4 agonist (SP-LPS). They showed higher in vivo antitumor activity and a higher percentage of activated immune cells in the TME than the Taxol-treated group.(Zheng D-W, 2016\)

Multipronged Approaches

Immunogenic chemotherapy has additionally been integrated with other ablative treatments, including PTT and PDT. Tao et al. created a versatile platform that integrates chemotherapy, photothermal therapy, and immunotherapy, made of DOX intercalated within CpG sequences, which were subsequently linked to gold nanorods. The nano system targeted gold rods to the tumor location for PTT, while the CpG stimulated an immune response, thereby augmenting the cytotoxic effects of DOX. The combination produced notable antitumor effectiveness and also fostered lasting tumor-specific immunity.(Tao Y, 2014)

The combined impact of chemotherapy with PDT to stimulate antitumor immunity, which may enhance the antitumor effectiveness of ICB. NCPs containing oxaliplatin (OxPt) in the core and PS pyrolipid in the outer layer were created for efficient chemotherapy and PDT, leading to a widespread tumor-targeted T-cell response. When used alongside anti-PD-L1, the NCP resulted in the regression of both the irradiated primary tumors and the non-irradiated tumors by promoting CD8+ and CD4+ T cell infiltration in bilateral mouse models of syngeneic colorectal cancer. Following this, Yang et al. co-encapsulated a PDT agent Ce6 and DOX into hollow H-MnO₂ nano-shells modified with PEG. The acquired H-MnO₂-PEG/C&D disbanded at lower pH levels in the TME to deliver the payloads, while also breaking down tumor H₂O₂ to alleviate tumor hypoxia. Consequently, an impressive in vivo synergistic therapeutic outcome was attained via the integration of chemo-photodynamic therapy and the ensuing antitumor immune reaction. Additional integration with ICB resulted in tumor suppression at remote locations, indicating potential for addressing tumor metastases.(He C, 2016)

Drawbacks

Despite promising potential, some major drawbacks are still associated with nanoparticle-mediated immunogenic cell death in cancer immunotherapy. In many cases, the design and preparation of delivery systems using nanoparticles involve complicated and costly processes that may impede their scaling-up and application in the clinic. There are also concerns over the potential for the long-term accumulation of nanoparticles in organs and tissues, because they cannot always be completely removed from the body. Diverse tumor microenvironments in different patients may lead to varying therapeutic responses, making it difficult to have uniform treatment protocols. In addition, there are regulatory issues and safety concerns in the use of new nanoparticle formulations, especially regarding their potential immunogenicity and toxicity properties. The dimensions, surface chemistry, and targeting ligands of nanoparticles are challenging to optimize, as these play a significant role in their biodistribution and

therapeutic efficacy. Additionally, lack of adequate long-term safety data and the risk of unpredictable immune responses pose additional risks that have to be carefully weighed before it can be used clinically in a large scale.

Future perspectives

Cancer immunotherapy has shown exciting clinical responses owing to its unique advantages, such as the induction of specific antitumor immunity and longterm immunological memory response, but the low response rate and the potential side effects are still significant hurdles for the broad application of cancer immunotherapy in the clinic. This review illustrates that various established and new inorganic and polymeric nanoparticles, including AuNPs, MOFs, and micelles, have been assessed as ICD-inducing agents, capable of enhancing treatment results in conjunction with immunotherapies while minimizing systemic toxic effects. Nonetheless, the effectiveness of various nanoparticle platforms or applications cannot be easily assessed since each experiment was conducted separately. Additionally, further efforts are required to observe the dynamic immune response and comprehend the specific influence of each combinatorial strategy on the TME, aiming to offer justifications or direction for choosing the optimal combinatorial approach for each patient. In particular, it is crucial to clarify if a NP-mediated therapy is better suited for combination with an immune adjuvant or ICB. Furthermore, the timing of the ICD-inducing methods and immunotherapies may also influence the therapeutic effectiveness and requires further investigation. Up to now, only a limited number of stimuli have demonstrated the ability to cause true ICD; identifying additional compounds or methods that make cell death immunogenic is critically needed in clinical settings. To preferentially transport ICD-inducing agents to tumors, particularly metastatic ones, nanoparticles must be administered systemically. Nevertheless, the majority of current studies have employed i.t. delivery, likely due to issues with stability, poor tumor accumulation, or significant toxicity; therefore, there is an urgent need for the development of novel nanoparticle platforms that can improve tissue localization and response

following systemic administration.

Conclusion

In conclusion, nanoparticle-facilitated ICD represents a hopeful new area in cancer immunotherapy that provides multiple benefits compared to traditional approaches. Our analysis of the existing literature shows that nanoparticle delivery systems can greatly improve the immunogenic response to cancer cell death while reducing systemic toxicity. Preclinical studies have demonstrated significant potential in the strategic development of nanoparticles to aim at particular cellular pathways and activate specific ICD mechanisms. The integration of ICD-inducing agents into nanocarriers greatly improves their therapeutic index, enabling more accurate targeting of tumor cells and safeguarding healthy tissue. The characteristics of controlled release in nanoparticle systems allow for prolonged exposure to ICD inducers, which could result in stronger immune responses. Furthermore, the adaptability of nanoparticle platforms allows for the simultaneous delivery of several therapeutic agents, enhancing the synergistic effects among various immunotherapy methods. Translating promising preclinical results into clinical success will necessitate additional refinement of nanoparticle design, focusing on enhancements in biodegradability, targeting effectiveness, and scalable production methods. Additionally, a more comprehensive grasp of the intricate relationships among nanoparticles, the immune system, and the tumor microenvironment is essential for optimizing therapeutic results. In the future, the area would gain from, Standardized methods for assessing the ICD-inducing potential of nanoparticle systems, creation of predictive models for patient reactions to nanoparticle-based immunotherapy and investigating combination approaches that integrate nanoparticle-based ICD inducers with traditional immunotherapy methods. This is a developing area, with continuous advancement in the understanding of nanoparticles and their application for ICD induction. With further development of such understanding, this approach provides vast potential to advance the practices of cancer immunotherapy and, hence, enhance the quality of life for

patients. Future research endeavors should address the above challenges and also broaden innovative applications in personalized medicine strategies.

Conflict of Interest

Authors declare no conflict of interest.

Acknowledgement

Authors are indebted to Swami Vivekananda University for providing support in order to complete this project.

References

- Amrolia, P. J., & Pule, M. (2015). Chimeric antigen receptor T cells for ALL. *The Lancet*, 385(9967), 488-490.
- Celli, J. P., Spring, B. Q., Rizvi, I., Evans, C. L., Samkoe, K. S., Verma, S., ... & Hasan, T. (2010). Imaging and photodynamic therapy: mechanisms, monitoring, and optimization. *Chemical reviews*, 110(5), 2795-2838.
- Chatterjee DK, Fong LS, Zhang Y, *Adv. Drug Delivery Rev* 2008, 60, 1627 – 1637;
- Chen, D. S., & Mellman, I. (2013). Oncology meets immunology: the cancer-immunity cycle. *immunity*, 39(1), 1-10.
- Chen, T., Guo, J., Han, C., Yang, M., & Cao, X. (2009). Heat shock protein 70, released from heat-stressed tumor cells, initiates antitumor immunity by inducing tumor cell chemokine production and activating dendritic cells via TLR4 pathway. *The journal of immunology*, 182(3), 1449-1459.
- Dranoff, G. (2004). Cytokines in cancer pathogenesis and cancer therapy. *Nature Reviews Cancer*, 4(1), 11-22.
- Evans, S. S., Repasky, E. A., & Fisher, D. T. (2015). Fever and the thermal regulation of immunity: the immune system feels the heat. *Nature Reviews Immunology*, 15(6), 335-349.
- Gubin, M. M., Zhang, X., Schuster, H., Caron, E., Ward, J. P., Noguchi, T., ... & Schreiber, R. D. (2014). Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature*, 515(7528), 577-581.

Homma, T., & Fujii, J. (2016). Heat stress promotes the down-regulation of IRE1 α in cells: An atypical modulation of the UPR pathway. *Experimental cell research*, 349(1), 128-138.

Mason, K. A., & Hunter, N. R. (2012). CpG plus radiotherapy: a review of preclinical works leading to clinical trial. *Frontiers in oncology*, 2, 101.

Moy, A. J., & Tunnell, J. W. (2017). Combinatorial immunotherapy and nanoparticle mediated hyperthermia. *Advanced drug delivery reviews*, 114, 175-183.

Restifo, N. P., Dudley, M. E., & Rosenberg, S. A. (2012). Adoptive immunotherapy for cancer: harnessing the T cell response. *Nature Reviews Immunology*, 12(4), 269-281.

Ribas, A., & Wolchok, J. D. (2018). Cancer immunotherapy using checkpoint blockade. *Science*, 359(6382), 1350-1355.,

Slovak R, Ludwig JM, Gettinger SN, Herbst RS, Kim HS, J. Immunother. Cancer 2017, 5, 78.

Su, B., Cengizeroglu, A., Farkasova, K., Viola, J. R., Anton, M., Ellwart, J. W., ... & Ogris, M. (2013). Systemic TNF α gene therapy synergizes with liposomal doxorubicine in the treatment of metastatic cancer. *Molecular Therapy*, 21(2), 300-308.

Tao, Y., Ju, E., Liu, Z., Dong, K., Ren, J., & Qu, X. (2014). Engineered, self-assembled near-infrared photothermal agents for combined tumor immunotherapy and chemo-photothermal therapy. *Biomaterials*, 35(24), 6646-6656.

Zhao, X., Yang, K., Zhao, R., Ji, T., Wang, X., Yang, X., ... & Nie, G. (2016). Inducing enhanced immunogenic cell death with nanocarrier-based drug delivery systems for pancreatic cancer therapy. *Biomaterials*, 102, 187-197.

Zheng, D. W., Chen, J. L., Zhu, J. Y., Rong, L., Li, B., Lei, Q., ... & Zhang, X. Z. (2016). Highly integrated nano-platform for breaking the barrier between chemotherapy and immunotherapy. *Nano letters*, 16(7), 4341-4347.

Zitvogel, L., Kepp, O., Senovilla, L., Menger, L., Chaput, N., & Kroemer, G. (2010). Immunogenic tumor cell death for optimal anticancer therapy: the calreticulin exposure pathway. *Clinical Cancer Research*, 16(12), 3100-3104.

He, C., Duan, X., Guo, N., Chan, C., Poon, C., Weichselbaum, R. R., & Lin, W. (2016). Core-shell nanoscale coordination polymers combine chemotherapy and photodynamic therapy

to potentiate checkpoint blockade cancer immunotherapy.
Nature communications, 7(1), 12499.