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Controlling Melanoma at the Local and Systemic Level: Is a Combination of Ablative Therapy and Immunotherapy the Way Forward?

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Although substantial progress has been made in developing treatments for primary tumors, the main challenge in cancer therapy remains the treatment of metastatic disease. Importantly, a number of primary treatment modalities have been shown to induce an anti-tumor immune response. One can thus envision a treatment strategy where ablation of a primary tumor in combination with immunotherapy can be used to modulate the immune response and direct it against metastatic sites. To that end, recent studies have shown that nanomaterials are effective tools for tuning immunity by facilitating delivery of antigens and adjuvants to lymphoid tissues[1]. Therefore, we posit that local cancer treatments can be combined with nanoparticle-delivered immune therapies to generate systemic immune responses and treat metastatic disease.

Ablative treatments such as photodynamic therapy (PDT), radiofrequency ablation (RFA), hyperthermia, and gold nanoparticle (AuNP) meditated photothermal therapy (PTT) have been shown to cause tumor-specific immune responses[2]. In general, the immune response is generated by cancer cell death and the release of Damage Associated Molecular Patterns (DAMPs) and tumor antigens, which are then captured by dendritic cells (DCs) and macrophages[2,3]. The treatments also induce a strong inflammatory response, characterized by the release of cytokines such as TNF-α, IL-6, and IL-1β. Upon antigen capture the immune system can mounts an antigen specific immune response against distant sites, thus making normally local treatments that depend on tumor accessibility applicable to metastatic disease[2,3].

A number of studies have explored how to harness this response by testing combination immunotherapies. For instance, Castano *et al*. applied PDT in combination with low dose cyclophosphamide in a reticulum cell sarcoma model. The addition of low dose of cyclophosphamide depleted immune suppressive regulatory T cells (T_{reg}), thereby enhancing the immune response following PDT and promoting long-term survival in 70% of mice[4]. Gameiro et al. in turn, combined RFA with a poxviral vaccine against the

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carcioembryonic antigen (CEA) for treatment in a colon carcinoma tumor model. The group observed significantly higher proliferation of CEA-specific T cells with the combination therapy when compared to the individual treatments alone[5]. Similarly, Liu and colleagues combined RFA with heat shocked tumor cell lysate pulsed DCs to promote immunity against tumor recurrence [6]. In another strategy, Chen and colleagues applied granulocyte-macrophage colony stimulating factor (GM-CSF) administration and cytotoxic T lymphocyte associated antigen-4 (CTLA-4) blockade in conjunction with hyperthermia[7]. Although the immune mechanisms were not explored, the addition of GM-CSF is intended to promote DC recruitment and maturation while the CTLA-4 blockade is aimed at inhibiting T_{reg} activity. The combination induced a tumor-specific immune response mediated by natural killer (NK) cells, $CD8^+$, and $CD4^+$ T cells, and it inhibited tumor growth at distant established sites. The CTLA-4 blockade has also been applied in combination with cryoablation, again causing $CD8^+$ and $CD4^+$ T cell infiltration at secondary tumors and increasing the ratio of cytotoxic T cells to regulatory T cells at those sites [8].

Importantly, Li et al. have applied these combination concepts in a clinical study, combining photohermal therapy with the imiquimod immune adjuvant for *in situ* photoimmunotherapy (ISPI) of metastatic melanoma[9]. The treatment induced a complete response in six out of eleven patients and resulted in a 12-month survival probability of 70%. The clinical application of local ablation to induce systemic immune response is thus viable, but further research into the exact immune mechanisms and the methods by which to modulate them is still necessary.

Bear and colleagues characterized the immune effects of AuNPmediated PTT and explored this modality in combination with adoptive T cell therapy[10]. Like other ablative treatments, PTT is limited in that it can only be applied to accessible sites and thus has not been used in a metastatic context. By examining the immune response that follows, however, Bear et al. found that PTT induces an immune response with anti-tumor activity. Importantly, however, the anti-tumor activity appeared dependent on distant tumor location; tumors located subcutaneously shrunk while those in the lungs grew after PTT of a primary site. This growth appeared to be induced by an inflammatory response to PTT that caused a systemic increase in immune suppressive myeloid-derived suppressor cells (MDSCs), and this effect was counter-acted with the combination with adoptive T cell therapy[10]. These results therefore indicate that the immune response to local treatments is complex, and potential combinations can be tailored to address both immune stimulatory and suppressive elements.

It is therefore important to further understand the immune response that follows a local ablative treatment and the immune effector cells that are involved. Clearly, cells such as DCs and macrophages are critical to the initial response and to inducing T cell activity against other sites. However, immune suppressive cells such as tumor-associated macrophages (TAMs), T_{regs}, and MDSCs can inhibit the response to local treatment and should be targeted as well. Nanoparticles are naturally cleared by various immune populations and can thus serve as promising carriers for immune modulating agents[1]. For instance, particles have been utilized for antigen and adjuvant delivery to stimulate DCs and

other innate cells. Kwong and colleagues used liposomes to deliver a combination therapy of the CpG oligonucleotide and the CD40 monoclonal antibody, ultimately improving therapeutic effect and reducing systemic inflammation[11]. The group also showed that the liposomes localized the delivery of agents to DCs and macrophages in the tumor-proximal lymph node. Other studies have also demonstrated that nanoparticles enhance the efficacy of delivered treatments such as antigens [12] and adjuvants[13,14]. Niikuraet al. evaluated vaccine delivery with various AuNP designs and found that spherical AuNPs had an adjuvant effect and promoted increased antibody production. The particles also enhanced the inflammatory response in bone marrow-derived DCs *in vitro*[15]. Therefore, such nanoparticle-mediated treatments can be applied in combination with local treatments in order to boost the response of DCs and macrophages infiltrating the treated area.

Nanoparticle distribution within suppressive populations has also been explored, indicating that nanoparticles can be used to deliver immune modulating agents to suppressive cells. Kourtis *et al*., for example, have shown that intradermally administered pluronic-stabilized poly(propylene sulfide) nanoparticles target MDSCs in the draining lymph nodes and spleens of tumor bearing mice. The researchers also observed significant particle uptake within MDSCs of the tumor microenvironment[16]. Our group, in turn, has shown that polyethylene glycol (PEG) coated AuNPs administered intravenously distribute mainly in B cells, granulocytes, and dendritic cells in the spleen, with a portion of the dose also associating with MDSCs[17]. Further research into the nanoparticle characteristics that influence distribution within immune cells will permit better targeting of immune populations. Sacchetti et al., for example, have developed single walled carbon nanotubes (SWCNT) targeted toward the glucocorticoid-induced TNFR-related receptor (GITR) in T_{regs}. Their work showed that the SWCNT could be specifically targeted to T_{regs} in the tumor as opposed to non-regulatory T cells or T_{regs} in the spleen[18]. This level of targeting to such suppressive populations then paves the way for delivery of agents that can polarize the cells' activity.

The ablative treatment of primary sites can generate strong anti-tumor immune responses, yielding an opportunity to not only eliminate an accessible tumor but also treat distant metastases. There are numerous treatment modalities that can be exploited for this purpose, but future studies should characterize the immune responses to each treatment. In general, the immune system responds to cancerous cell death and the subsequent release of DAMPs and cancer antigens. Further characterization will elucidate the immune populations driving the systemic response, including any suppressive mechanisms that may be involved. Researchers can then develop combination strategies aimed at promoting the immune stimulatory cells and at inhibiting the suppressive ones, and nanotechnologies can be useful tools in developing combinatorial opportunities. A number of materials are non-toxic and have been shown to accumulate in immune cells in the spleen and lymphatic system. Nanoparticles can be easily synthesized and functionalized with targeting ligands and immune modulating agents, and they have been shown to enhance the activity of such agents. Therefore, the fields of nanotechnology and immunology would benefit from closer collaboration, allowing researchers to develop materials that can deliver cargo to specific

immune populations. The ensuing nanoparticle mediated immunotherapies can then be applied in combination with local therapies for the treatment of metastatic disease.

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