Immuno-Oncology Technology 2 (2019) 11–13

Contents lists available at ScienceDirect

Immuno-Oncology Technology

journal homepage: www.esmoiotech.org

Technology Explained

Sprayable gel for postsurgical immunotherapy

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ARTICLE INFO

Keywords:
Surgery
Cancer immunotherapy
Local treatment
Tumor recurrence

ABSTRACT

Background: Surgery remains the first option to treat most solid tumors. However, despite the development of surgical techniques, the elimination of tumor recurrence after surgery remains a challenge.
Design: In a recent study published in *Nature Nanotechnology*, we described an *in-situ*-sprayed gel for local delivery of bioresponsive and immunotherapeutic calcium carbonate nanoparticles encapsulated with anti-CD47 antibodies (aCD47@$CaCO_3$) to the surgical site after surgery. $CaCO_3$ nanoparticles react with H^+ in the surgical wound site, eliciting an immunosupportive tumor microenvironment after surgery. Meanwhile, the subsequently released aCD47 blocks the 'don't eat me' signal expressed on cancer cells to increase the phagocytosis of cancer cells by macrophages and activate T-cell-mediated antitumor immune responses.
Conclusion: The engineered immunotherapeutic gel could activate both innate and adaptive immune responses systemically after local treatment, effectively destroying the remaining cancer cells and reducing tumor recurrence.

Surgical resection remains the first option to treat most solid tumors [1]. However, even after resection of the primary tumor tissues, it remains challenging to eliminate residual microtumors and circulating cancer cells [2,3]. Perioperative inflammation induced by trauma poses a high risk for the development of tumor recurrence, accelerating local remaining tumor relapse as well as promoting tumor invasion and metastasis [4–6]. Thus, additional treatments including chemotherapy and radiotherapy are usually applied after surgery. Unfortunately, therapeutic efficacy is still limited [7], as even a very small number of remaining tumor cells could result in the regrowth of tumor tissues at local or distant sites.

Cancer immunotherapy by educating and awakening a patient's own immune system against cancer cells has been progressing rapidly, and exhibits tremendous promise for the next generation of cancer treatments [8–11]. Macrophages, an important component of innate immunity, are important for the uptake of cells that are damaged, superfluous and/or cancerous. Phagocytosis of 'self' cells is a tightly regulated multifactorial process, partially governed by the 'self-signal' protein on their surface including CD47 [12,13]. However, cancer cells can up-regulate the antiphagocytic 'don't eat me' signal from inflammatory trauma after surgery, thus avoiding phagocytosis by macrophages [14,15]. The immunotherapeutic antibody anti-CD47 is able to neutralize CD47; activate phagocytic cells including macrophages, dendritic cells and neutrophils; and further activate the adoptive immune response. Despite significant investment and remarkable progress in phase I clinical trials, there is still potential for improvement of the current CD47 blockade strategy [16–19]. The occurrence of thrombocytopenia and anemia after systemic injection of CD47 antagonists usually limits their wide application. Efforts to reduce these sideeffects and improve therapeutic effects are highly desirable in the field of CD47-mediated immunotherapy. In addition, an immunosuppressive tumor microenvironment (TME) can lead to the failure of immunotherapy [20,21].

In our recent work published in *Nature Nanotechnology*, we engineered a sprayable bioresponsive immunotherapeutic fibrin gel to inhibit tumor recurrence and metastasis after surgery (Figure 1) [22]. Briefly, biocompatible carbonate ($CaCO_3$) nanoparticles containing anti-CD47 were synthesized via biomineralization, and incorporated into a fibrin gel which has been approved previously by the Food and Drug

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https://doi.org/10.1016/j.iotech.2019.07.001

Available online 31 July 2019

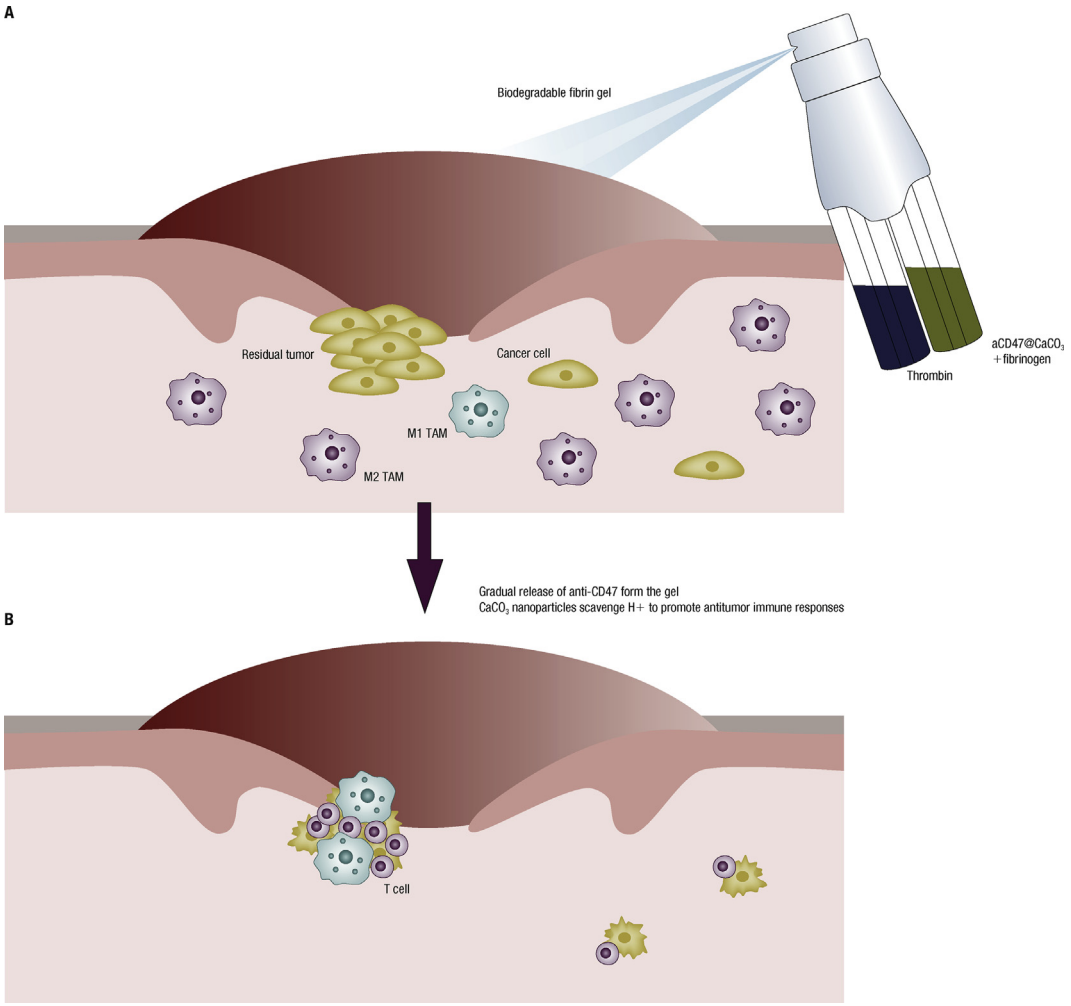

Figure 1. Schematic representation of *in-situ*-sprayed immunotherapeutic fibrin gel. (A) Following surgical resection of a tumor, biodegradable fibrin gel containing $CaCO_3$ nanoparticles encapsulated with the immunotherapeutic antibody anti-CD47 is sprayed on the wound. (B) Anti-CD47 is gradually released into the tissue, blocking CD47 on the surface of cancer cells, to increase the phagocytosis of cancer cells by macrophages and initiate T-cell-mediated antitumor responses. $CaCO_3$ nanoparticles also scavenge H^+ in the surgical wound site, eliciting an immunosupportive tumor microenvironment after surgery. TAM, tumor-associated macrophage.

Administration for wound healing [23,24]. The fibrin gel was formed by the interaction of fibrinogen and thrombin, and can be sprayed on surgical wounds for quick and convenient treatment, promoting wound healing by forming a connective, protective layer over the injured tissue. The fibrin-gel-based delivery platform enhanced the local retention of immunotherapeutics, improved their effect on tumor-infiltrating lymphocytes (TILs), and reduced the toxicity resulting from leakage into the systemic circulation. $CaCO_3$ nanoparticles were expected to dissolve gradually, releasing the encapsulated anti-CD47 into the acidic inflammation and TME, which usually plays an immunosuppressive role by affecting immune cell functions including the polarization of tumor-associated macrophages (TAMs), T-cell proliferation and cytokine production. We found that the $CaCO_3$ nanoparticles relieved the adverse effects of the acidic environment, such as promoting the activation of M1 type TAMs. Meanwhile, the released anti-CD47 blocked the interaction between CD47 and signal regulatory protein α, increasing macrophage phagocytosis of cancer cells and tumor antigen presentation to T cells, thus activating the adaptive immune response.

Utilizing fluorescent-dye-labelled anti-CD47, aCD47@$CaCO_3$ nanoparticles and aCD47@$CaCO_3$-loaded fibrin gel, we tracked the antibody signal over time, confirming the prolonged retention and gradual release of anti-CD47 from the gel over 3 weeks. As shown in the confocal imaging and flow cytometry results, the blockade of different cancer cells by the antibody significantly increased the phagocytosis of cancer cells by bone-marrow-derived macrophages. Moreover, we demonstrated that CD47 blockade could activate dendritic cells and macrophages *in vivo*, which usually play an important role in initiation of the adaptive immune response after effective phagocytosis and presentation of antigens.

Application of aCD47@$CaCO_3$-loaded fibrin gel to the tumor resection site effectively activated both the innate and adoptive immune responses, improving antitumor efficiency and decreasing the potential for tumor recurrence. This prolonged the survival of mice without the induction of side-effects. Moreover, local treatment with aCD47@$CaCO_3$-loaded fibrin gel was able to delay the growth of distant tumors due to systemic immune responses induced by the local application of immunotherapeutic gel. Analysis of the mechanism of action confirmed that the number of M1-polarized TAMs and $CD103^+$ dendritic cells increased significantly in the relapsed tumors after treatment. Of note, the infiltration of TILs, especially $CD8^+$ T cells, increased in both relapsed and distant tumors, and the infiltration of $CD4^+$ T cells also increased but not significantly. Increased TILs in distant tumors may be attributed to activated macrophages and dendritic cells, which phagocytosed cancer cells and presented tumor-specific antigens to $CD8^+$ T cells, triggering the robust antitumor immunity. Thus, local treatment with the immunotherapeutic gel activated the innate and adaptive immune responses both locally and systemically. The local and systemic immune effects were further improved, including a significant decrease in tumor growth, by combination with T-cell-activating antibodies, such as anti-PD-1

antibody.

Considering the ease of administration, good biocompatibility and the positive therapeutic results of this technology, this method shows potential for translation into clinical use. Further validation of the results is needed in large animal models, which may help to define the feasibility of this strategy. Specifically, we need to determine appropriate dose levels and optimize the release kinetics of immunotherapeutics to improve outcomes including overall survival, progression-free survival and disease-free survival. More comprehensive evaluation of the potential side-effects is also important. As CD47 inhibitors have shown side-effects after systemic administration in clinical trials, such as thrombocytopenia and anemia, it is important to investigate whether local application could cause toxicity in patients and define its dose-dependent relationship. All mouse studies were carried out following the protocols approved by the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill and North Carolina State University and complied with all relevant ethical regulations.

Funding

This work was supported by grants from the Jonsson Comprehensive Cancer Center, United States; UCLA, United States; Alfred P. Sloan Foundation, United States; NC TraCS, United States; Collaborative Innovation Center of Suzhou Nano Science and Technology, China; Priority Academic Program Development of Jiangsu Higher Education Institutions, China; 111 Project, China.

Disclosure

The authors have declared no conflicts of interest.

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