Modeling Immune Checkpoint Inhibitors

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Immune checkpoint inhibitors are revolutionizing the treatment of cancer, but models that accurately predict their efficacy before administering them to humans are badly needed. This entry presents the application of a microfluidic tumor model that simulates the interactions between immune cells and tumors in a dynamic microenvironment, utilizing real-time imaging and image analytic algorithms to demonstrate excellent correlations between the laboratory model and animal studies. Future applications of the system in precision medicine will explore the use of the device for selecting patient-specific therapies for cancer.

Keywords: Immunology ; Microfluidics ; Oncology ; Immunotherapy ; Image Analytics ; Fluid Dynamics ; Lymphocytes ; Tumor Microenvironment

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The emergence of monoclonal antibodies that target immune checkpoint pathways is one of the most promising developments in the recent history of cancer treatment, with extraordinary clinical responses observed for particular groups of patients and specific types of cancers. A pioneering discovery in medicine was the realization that tumors often evade attack by the immune system by dysregulating the normal function of immune checkpoint pathways, and that the use of specific immune checkpoint inhibitors (ICIs) can effectively block these mechanisms of immune resistance.

1. Introduction

Immune checkpoint inhibitors target specific pathways such as CTLA4 and PD-1, and have shown remarkable success against melanoma[1], non-small-cell lung cancer[2], and Hodgkin's lymphoma[3], with mixed results in several other cancers. Since their approval in 2014, two immune checkpoint inhibitors have vaulted into the ten top-selling prescription drugs in the world, and approvals for new drugs and applications to additional types of cancer continue to mount. In spite of these dramatic successes, further progress is limited by additional resistance pathways and the potential need for involvement of multiple checkpoint inhibitors. Exploration of these pathways and mechanisms would benefit from preclinical models with higher throughput; systems that can shed light on underlying mechanisms of resistance, response, and off-target effects; and technologies capable of directly evaluating human tumor tissue. These requirements have spurred efforts toward the development of model systems that recapitulate key aspects of the tumor microenvironment and that can be used to screen responses to emerging ICIs and combination therapies, which will be critical to future advances in immunotherapy[4].

Models of the tumor microenvironment have been under development for more than a decade[5][6], leveraging early pioneering work in microfluidics[7][8][9] and, more recently, microphysiological systems[10]. Many of these developments involve two-dimensional culturing of cancer cell lines, suspended spheroid systems, and organoids cultured in extracellular matrix[11] for the purposes of drug screening[12][13] and elucidating the mechanisms involved in the immune checkpoint blockade[14][15]. However, these models do not faithfully recapitulate the in vivo tumor microenvironment due to alterations in the cellular and matrix composition that may limit their predictive power compared to studies on unmodified tumor fragments[16]. Microfluidic platforms for tumor slices [17][18][19][20] and bioprinting technologies have also emerged[21], as have systems that model angiogenesis, metastasis, extravasation, and other phenomena[22][23][24][25][26][27][28][29][30]. While these systems have shown utility in modeling chemotherapeutic responses and immune checkpoint behavior, most are non-perfused systems that do not capture the dynamics of lymphocyte migration and drug transport[31], and their study is often of limited duration due to a rapid decline in viability beyond 24–72 h[32][33]. An urgent need remains for engineered platforms capable of supporting viable tumor fragments over a longer time frame, accommodating dynamic interactions between lymphocytes and tumors, and enabling real-time imaging in order to quantify tumor killing and lymphocyte infiltration in response to ICI treatments.

Researchers report on the application of a perfused microfluidic platform technology, termed EVIDENT (ex vivo immuno-oncology dynamic environment for tumor biopsies), to assess the efficacy of two classes of ICIs against three syngeneic mouse tumor models exposed to flowing drug-treated lymphocytes.

The system has been previously reported and shown to recapitulate tumor killing induced by anti-PD-1 treatment of tumor-infiltrating lymphocytes (TILs) with the known responder MC38 syngeneic mouse model[34]. Dynamic perfusion of unmodified tumor fragments permits the preservation of viability over periods of 7 days or more, and enables interactions between flowing TILs and spatially entrained tumor tissues. The system is constructed using materials chosen to minimize the drug adsorption phenomena associated with commonly used microfluidic substrates such as poly(dimethylsiloxane) [35], while still providing a high degree of optical transparency for clear image quality[36]. Further, mechanisms of TIL infiltration, migration, and lymphocyte-mediated killing within tumor fragments, which cannot be observed directly in animal studies, are readily accessible in real time during the course of an EVIDENT experiment. These advantages render the EVIDENT system a powerful tool for increasing the efficiency of checkpoint blockade studies, reducing the number of animals required to perform such investigations, and providing mechanistic insights into the key immune cell–tumor interactions.

2. challenges and perspectives of modeling immune checkpoint blockade

It is important to acknowledge that many questions and challenges remain in modeling immune checkpoint blockade. These early investigations are only the beginning of the development of the complex tools necessary to explore the full range of key immune–tumor interactions involved in the process. For instance, the transition of technologies such as EVIDENT from syngeneic mouse tumor platforms to human tumor samples is quite challenging, due to the highly heterogeneous nature of human tumors and the variable features present in biopsied samples from patients. Additional complexity relates to efforts to capture the complexity of the immune response in a relatively simple system; it remains unclear to what degree response to ICIs is driven by interactions between checkpoint compounds and TILs present in the tumor relative to effects on circulating lymphocytes distant from tumor loci. Recent evidence suggests that the latter represents perhaps the predominant mechanism for ICI response[37], and therefore the next generation of immuno-oncology models may be required to incorporate circulating immune cells in order to accurately represent the process. Toward this end, future studies with the EVIDENT system might incorporate non-TIL immune-cell populations derived from companion blood samples from mice or humans, and may evaluate the tumor-killing response as a function of the numbers of TILs or other immune cells reaching the tumor fragments. Such experiments present challenges due to greater complexity in the immune component of the system and in quantification of the immune cell populations involved. In spite of these and other challenges, this early investigation provides encouraging evidence that rapid assessment of ICI efficacy can be recapitulated ex vivo in a model system as a powerful tool for future drug development and precision medicine.

Results provided by the EVIDENT system and described here may be assessed in reference to alternative in vitro or ex vivo methods for evaluation of the efficacy of chemotherapeutic compounds or ICIs, all of which have the potential to provide higher throughput and more rapid indications of drug response than in vivo studies Promising results have emerged from studies with tumor spheroids, integrated in some cases with flow and immune cells[14], but these face challenges associated with recapitulating the tumor microenvironment. Significant progress has been made toward integrating microfluidic perfusion systems with tumor slices[19]; however, the duration of these studies is limited and challenges with integrating immune components remain. In this context, the EVIDENT system represents a potential path toward high-throughput dynamic models that integrate immune cells and perfusion flow with tumor fragments closely representative of the in vivo microenvironment. Future applications of this system might explore the relative roles in tumor killing of immune cells derived from various sources, comparing the effects of circulating TILs as in this study with naïve T cells obtained from the blood and introduced into the circulation in the EVIDENT system.

Finally, there are broader potential impacts of this technology on the field of immuno-oncology, ranging from the ability to probe mechanistic phenomena at the cellular level in dynamic studies to rapid assessment of combination therapies, and ultimately in the context of precision medicine placed directly in the clinic. Tools such as EVIDENT can bridge the gap between current high-throughput in vitro screens and animal studies to reduce and more precisely target the latter stage in preclinical development. For instance, the EVIDENT study required a much smaller number of mice than would a comparable in vivo study. In clinical applications, the small size of EVIDENT fragments will enable sampling of tumor biopsies at negligible risk for the patient. The benefit, a highly predictive and timely estimation of the most efficacious treatment protocol, has the potential to allow identification of the best suited therapy regimen for the individual patient at a specific stage of the disease.

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