Intratumoral delivery of mRNA: Overcoming obstacles for effective immunotherapy

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Abbreviations: CTLs, cytotoxic T lymphocytes; DCs, dendritic cells; Fβ2, a fusokine consisting of IFNβ, fused to the ectodomain of the TGFβ receptor II; IMP, investigational medicinal product; MDSCs, myeloid-derived suppressor cells; TAAs, tumor-associated antigens; TiDCs, tumor-infiltrating DCs; TME, tumor microenvironment

The immunosuppressive tumor microenvironment (TME) is a major obstacle in cancer immunotherapy. Therefore, it has gained attention as a target site. mRNA emerged as a versatile drug class for cancer therapy. We reported that intratumoral administration of mRNA encoding the fusokine Fβ2 supports tumor-specific T-cell immunity. This study provides proof of concept of the use of mRNA to modulate the TME.

Cancer Immunotherapy: Opportunities and Obstacles

The idea that the immune system can be exploited to combat cancer originated in the nineteenth century when it was observed that tumors occasionally shrunk when infected.1 Ever since, scientists have studied the immune system, searching for a means to harness the body’s defense mechanisms against cancer. Breakthroughs such as the identification of tumor-associated antigens (TAAs), dendritic cells (DCs), major histocompatibility complex (MHC) I-restricted antigen presentation to CD8+ T cells, and the production of synthetic antibodies have shaped the cancer immunotherapy field. Based on these findings, therapies such as cancer vaccines, adoptive T-cell transfer, and antibodies were developed and these are now extending the lives of patients.2 Although the number of patients that benefit from these therapies is growing, there are still a number of obstacles to overcome.

A major hurdle is the immunosuppressive tumor microenvironment (TME). Here, tumor cells and immune cells such as myeloid-derived suppressor cells (MDSCs), macrophages, and regulatory T cells cooperate to dampen antitumor immune responses using a plethora of inhibitory mechanisms. Several drugs have been developed to revert the suppressive TME, including pattern-recognition receptor agonists, stimulatory cytokines, decoy receptors that capture immunosuppressive cytokines, and monoclonal antibodies that target immune-checkpoint molecules. The targets of these immunomodulatory drugs can be found within the TME.3,4

Intratumoral Delivery of Immunomodulatory Drugs

In 1890, William Coley injected bacterial toxins into primary tumors, showing tumor regression in a number of patients.1 Nonetheless, for decades, drugs were preferentially administered systemically, because such administration was intended to induce strong systemic antitumor immune responses capable of rejecting primary and metastasized tumors.5 However, the limitations encountered with systemic delivery of immunomodulatory drugs, of which toxicity is the most pressing, together with the growing appreciation that many of their targets are present within the TME has revived the concept of intratumoral therapy delivery.

A multitude of studies analyzing the activation of cytotoxic T lymphocytes (CTLs) and inhibition of regulatory factors evidenced that local delivery of cancer immunotherapies has several advantages. These include stimulation of systemic immune responses with enhanced breadth and simultaneous reduction of immunosuppression that, together, enable therapeutic antitumor immunity with little or no toxicity.4 The broad effects elicited by single agents are explained by the intricate communication between cells and the suppressive mechanisms they exert in the TME. This implies that the modulation of 1 cell population or suppressive mechanism also impacts others.

mRNA: An Interesting Technology Platform for Intratumoral Therapy

Weide et al.6 were the first to inject naked tumor mRNA into the dermis of melanoma patients, showing increased humoral immune responses in several patients. This pioneering work has put mRNA on the map as a promising drug class for cancer immunotherapy. Ever since, in vitro-transcribed mRNA has been evaluated as an investigational medicinal product (IMP) for the delivery of TAAs...
Importantly, mRNA as an IMP is and/or cell-reprogramming proteins into DCs.\(^7,8\) Therefore, delivering immunomodulatory drugs such as antibodies, cytokines, and decoy receptors under the form of mRNA represents an attractive approach that circumvents the cumbersome time- and money-consuming approach involved in producing recombinant proteins according to Good Manufacturing Practices regulations.

A prerequisite for the use of mRNA (as IMP) to modulate the TME is its uptake and translation by cells within the tumor. We demonstrated, in several mouse tumor models using mRNA encoding firefly luciferase, that mRNA can be delivered to the tumor and the expression of firefly luciferase can be detected for up to 5 d post-delivery. Moreover, we demonstrated, by using the Baf3\(^{-/-}\) model, that CD8\(^{+}\) cross-presenting DCs are mainly responsible for the uptake of naked mRNA.\(^9\) This finding opens the possibility of exploiting tumor-infiltrating DCs (TiDCs) to produce immunomodulating proteins locally.

As a proof of concept, we delivered mRNA encoding a fusokine consisting of interferon \(\beta\) (IFN\(\beta\)) fused to the ectodomain of the transforming growth factor \(\beta\) (TGF\(\beta\)) receptor II, referred to as F\(\beta\)\(^2\). The rationale was that IFN\(\beta\) would exert an immunostimulatory function, whereas the ectodomain of the TGF\(\beta\) receptor II would reduce the TGF\(\beta\)-mediated immunosuppression. We showed that F\(\beta\)\(^2\) reduced the suppressive activity of MDSCs, while it enhanced the stimulatory capacity of DCs and the lytic activity of CTLs. Moreover, F\(\beta\)\(^2\) enhanced the expression of MHC I on tumor cells, thus enhancing recognition and killing by CTLs. Nonetheless, delivery of F\(\beta\)\(^2\) mRNA to the tumor only resulted in a transient delay in tumor growth. Further analysis showed that F\(\beta\)\(^2\) induced a high expression of PD-L1 on tumor cells and that a combination of F\(\beta\)\(^2\) mRNA delivery and PD-1/PD-L1 blockade enhanced the potential of this local therapy (Fig. 1).\(^{10}\)

To our knowledge, this is the first study showing the use of mRNA encoding secreted proteins to engineer the TME. The finding that TiDCs can be exploited as local producers of mRNA-encoded proteins opens new avenues to deliver stimulatory cytokines, decoy receptors, or combinations thereof, as well as molecules developed to block inhibitory or to activate stimulatory immune checkpoints. In addition, the uptake of mRNA by TiDCs offers the opportunity to develop antigen-independent immunization strategies, as TiDCs carry TAAs. Stimulating TiDCs to drain to lymph nodes and activate CTLs, for example, through the delivery of TriMix mRNA — a mix of 3 mRNA molecules encoding the CD40 ligand, constitutively active TLR4, and CD70 — is an attractive strategy.\(^9\) In addition mRNA-mediated TiDC-stimulation followed by mRNA-based engineering of the TME could be an attractive approach to activate CTLs and preserve their function in the tumor nest.

**Conclusion**

The growing appreciation of intratumoral delivery of cancer immunotherapy agents, as pioneered by William Coley in the nineteenth century, together with the revival of the use of mRNA as an anticancer drug, as pioneered in the clinic by Weide et al. in the twentieth century, will most likely lead to better outcomes with anticancer therapy in the 21st century.

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No potential conflicts of interest were declared.

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