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Cancer Therapy Using Lipid Nanoparticles to Encapsulate mRNA Mixtures Encoding Cytokines

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Abstract:

Compared with conventional cancer treatments such as chemotherapy and radiation therapy, mRNA gene therapy is a novel and promising approach. mRNA gene therapy has the advantage of being faster compared to other gene therapies and at a relatively low cost. Cytokines are potent modulators of the tumor microenvironment and were one of the first immunotherapies used to treat human cancers. The mRNA-generated cytokines have potential advantages over surface-engineered recombinant cytokines. Research has been showing effective therapeutic implications through local therapy by delivering immune-stimulated mRNAs to the tumor microenvironment (TME) with lipid nanoparticles (LNPs). Currently, new cytokine mRNA mixtures have been approved for their safe and efficient anticancer effects with low side effects, including a mixture of IL-12 and IL-27 mRNA, mRNA mixture encoding IL-23, IL-36, and OX40L, mRNA mixture encoding GM-CSF, IL15 sushi, IFNα, and scIL-12, etc. This review describes examples of enhancing efficacy through the use of LNPs to encapsulate mRNAs encoding cytokines and mRNA mixtures encoding two or more synergistic cytokines-lipid nanoparticles to improve therapeutic efficacy. Future research is required to consider translating *in vitro* and *in vivo* studies of mRNA mixture into clinical applications.

Keywords: mRNA; gene therapy; cancer; cytokines; immunotherapy.

1. Introduction

Cancers, as a life-threatening disease, can be induced by the uncontrolled division of cells, culminating in the formation of solid cells named tumors and liquid named cancers [1]. As a result, the development of effective cancer treatment technologies is receiving increasing attention. Traditional anticancer strategies tend to be through surgery, chemotherapy, and radiotherapy. Although they offer substantial benefits in eradicating primary tumors, the incidence of disease recurrence remains a common problem and is often cost-prohibitive [1]. Compared to these treatments, mRNA gene therapy is a novel and promising approach.

mRNA-based therapeutics have increased due to the fact that mRNA technology can generate a variety of vaccines and therapeutics in a short period, as well as its lower cost compared to conventional methods. In particular, the recent making of the SARS-CoV-2 mRNA vaccine stimulated interest in mRNA-based therapies, providing an effective boost to mRNA technology [2].

Cytokines can produce both autocrine and paracrine effects. Therefore, they can be considered potent regulators of the tumor microenvironment (TME) [3]. However, despite decades of clinical studies and clinical approvals of various recombinant cytokines, cytokines exhibited low therapeutic efficacy. The short half-life, the small treatment window, the need for multiple administrations, the adverse reactions induced by the necessary heavy doses, and the failure to achieve sufficient local concentrations are major obstacles to the applications of recombinant cytokines. Therefore, because their signaling activity can theoretically be maintained, mRNA-generated cytokines may perform better than traditional surface-engineered recombinant cytokines. Encapsulate mRNA-encoding cytokines by nanoparticles can prolong the half-life for systemic injection and in the TME and thus improve efficiency [4]. The treatment with mRNA mixtures containing two or more cooperating cytokines may induce a broader immune response to tumors compared to the encoding of a single cytokine by mRNA lipid nanoparticles (LNPs). The use of mRNA mixtures as a therapeutic approach has been utilized in several clinical trials for cancer treatment [4].

This review summarizes the use of cytokine mRNA mixtures as a method of tumor therapy and gives three examples to demonstrate its efficacy.

2. Cytokine mRNA Mixture as a Therapeutic Approach

Cytokines are integral components of adaptive and innate immune responses and were among the first immunotherapies used to treat human cancers [3]. Cytokines have been showing antitumor activities as they affect immune effector cells as well as stromal cells and enhance the recognition of tumor cells through cytotoxic effector cells. A number of cytokines are currently being explored in clinical trials, including interleukin-12 (IL-12), IL-15, IL-21, granulocyte-macrophage colony-stimulating factor (GM-CSF), and interferon α (IFNα) [5]. As research continues, these cytokines have been progressively used in the treatment of specific tumors. However, due to the somewhat narrow therapeutic scope, monotherapy cytokines are still unsatisfied.

Soluble cytokines usually play a role in a paracrine or autocrine manner over short distances and have relatively short half-lives [3]. Thus, once cytokines are given, they can be in large amounts in order to achieve adequate concentrations within the TME. However, since dose affects toxicity and excessive concentrations can cause severe toxic symptoms, especially flu-like symptoms, high concentrations of a single cytokine are not relevant in clinical trials [5].

A number of factors, such as concentration and toxicity limits the administration of monotherapy cytokines. Therefore, the use of combination therapy cytokines can reduce the concentration of each of these monotherapy cytokines to achieve a more meaningful and efficient tumor response.

3. Applications of Cytokine mRNA Mixtures

Cytokines are considered potent regulators of the TME because of their ability to produce autocrine and paracrine effects, but their systemic toxicity hampers the potent induction of antitumor immunity. In this context, local therapy by delivering immune-stimulated mRNAs to the TME through the use of LNPs is a promising method (Figure 1). LNPs have some special characteristics, such as easy manufacture of mass, sufficient encapsulation and delivery of mRNA, transient expression of proteins induced by mRNA, and small toxicity of intratumoral injection [6].

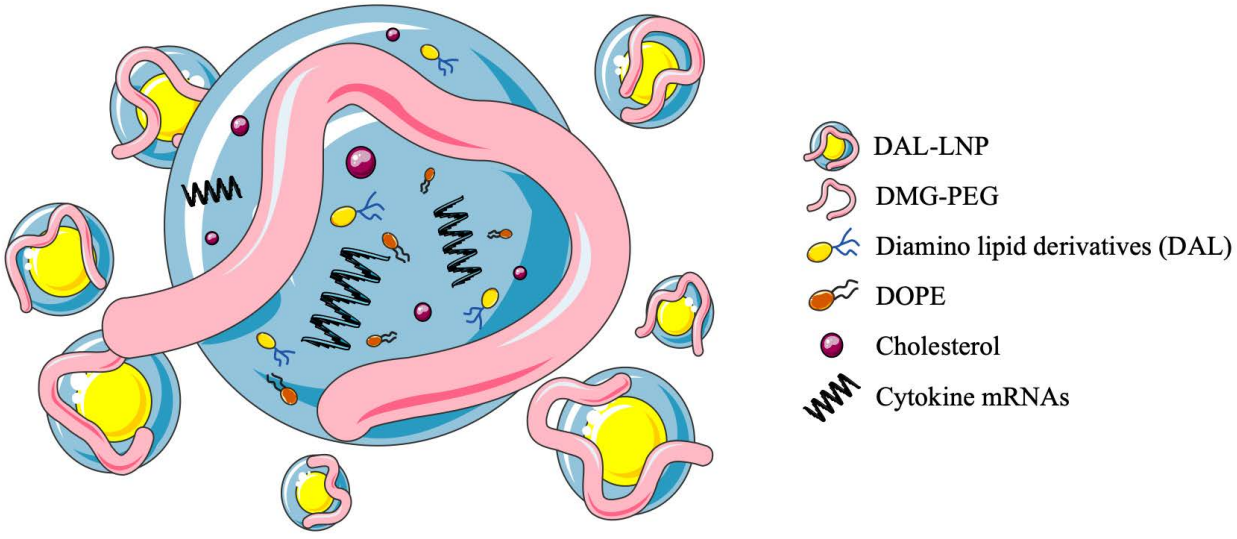

Fig. 1 Diamino lipid-derived nanoparticles (DAL-LNP) encapsulated with cytokine-encoding mRNAs. DMG, Dimethylglycine; PEG, Polyethylene glycols; DOPE, 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine. Figure credit: original.

Ideally, local immunotherapy can stimulate an immune response to tumors and avoid the toxicity related to systemic administration. In contrast to mRNA-LNPs encoding only a single cytokine, the use of mRNA mixtures-LNPs encoding two or more synergistic cytokines has a side antitumor immune response.

3.1 IL-12 and IL-27

In a mouse model of melanoma, intra-tumor delivery of mRNA LNPs encoding IL-12 and IL-27 induced potent infiltration of immune effector cells [4].

IL-12, a heterodimeric pro-inflammatory cytokine, can modulate T-cell and natural killer cell responses and enhance Th1/Tc1 responses to exhibit potent antitumor activity [7]. However, it may lead to fatal systemic toxic effects. Only if IL-12 is restrained to the TME can there be a reduction in systemic toxicity caused by this gene [6].

IL-27, an anti-inflammatory cytokine, has potent antitumor effects. IL-27 can increase the survival of T cells in the cellular microenvironment and enhance the generation of memory T cells through programming CD8+ T cells to a distinct T effector phenotype with increased IFN-γ as well as IL-10 [3].

The effects of every cytokine mRNA in diamino lipid DAL4 nanoparticles (DAL4-LNP) were compared by intra-tumor injection every other day. Different from DAL4-LNP encapsulated with IL-27 mRNA (DAL4-LNP-IL-27) or DAL4-LNP encapsulated by GM-CSF mRNA (DAL4-LNP-GM-CSF), DAL4-LNP encapsulated with IL-12 mRNA (DAL4-LNP-IL-12) had the best tumor-suppressing activity, the slower tumor growth and longer survival time. Therefore, intra-tumor injection of DAL-LNP to IL-12 and IL-27 mRNA promoted sustained inhibition of tumor growth and did not cause significant toxicity [6]

Combinations of IL-12 + GM-CSF mRNA, IL-12 + IL-27 + GM-CSF mRNA, and IL-12 + IL-27 mRNA in DAL4-LNP were comparatively tested to evaluate whether intratumoral injection of cytokine mRNA mixture LNPs induced better tumor growth inhibition. DAL4-LNP-IL-12 + IL-27 was superior to other mixture formulations in slower tumor growth and better survival and did not cause significant systemic toxicity at the doses at which it was tested [6].

3.2 IL-23, IL-36 and OX40L

Intratumorally administered LNPs encapsulating mRNA mixture with IL-23, IL-36, and OX40L may induce a potent antitumor response in TME. It leads to downstream cytokines and chemokines expression and inflammatory cell activation [4].

IL-23, which consists of the p40 subunit shared with IL-12 and the IL-23-specific p19 subunit, can act on Th1 effector/memory CD4+ T cells and promote the proliferation and IFN-γ production. IL-23 treatment has antitumor efficacy comparable to that of IL-12 but may be tolerated with improved efficacy compared to systemic IL-12 proteins [8]. IL-36, a member of the IL-1 cytokine family, is produced by innate immune cells and lymphocytes and induces the production of pro-inflammatory cytokines, chemokines, and co-stimulatory molecules that promote Th1 and Th17 cell polarization [9]. OX40L is a type II glycoprotein expressed predominantly on activated antigen-presenting cells (APCs) such as dendritic cells (DCs), activated B cells, and macrophages. OX40 agitation promotes T-cell responses and, in the case of topical therapies, may limit the response to antigens within the TME [10].

By testing single cytokines as well as IL-23 and IL-36γ dual mRNA treatments, comparisons yielded that among cytokines with single-agent efficacy, half of IL-23 induced complete tumor regression, whereas IL-36γ demonstrated delayed borderline tumor growth; IL-23 and IL-36γ dual mRNA therapy produced a powerful synergistic antitumor effect, and all tumors treated with this dual RNA drug were completely cured [10].

Dual therapy with IL-23/IL-36γ and IL-23/OX40L caused a CR, whereas the triple RNA mixture, including IL-23 and IL-36γ as well as OX40L, increased the CR rate to approximately 50% [10].

mRNAs and recombinant proteins have been compared in terms of antitumor efficacy. The total amount of target proteins in tumors injected with mRNA was calculated, and it was determined that the tumors were injected with an excess of recombinant proteins. The antitumor effect of mRNA injection was much better than that of IL-23/IL-36γ/OX40L-Fc protein injection, with a better survival rate [10].

3.3 GM-CSF, IL15 sushi, IFNα, and scIL-12

IL-15 fused with the sushi structural domain of the IL-15 is IL15-sushi. Intra-tumoral administration of saline-formulated N1-methyl pseudouridine (m1ψ)-modified mRNA mixtures, including mRNAs encoding GM-CSF, IL15-sushi, IFNα, and single chain IL-12 (scIL-12), was effective in inducing systemic antigen-specific T-cell amplification and immune memory formation [4].

IL-12sc is a fusion of the genes for the IL-12p40 and IL-12p35 subunits. GM-CSF is a small glycoprotein with a tetra-alpha-helical bundle structure. It can promote the survival of neutrophils, eosinophils, macrophages, megakaryocytes, and erythroid hematopoietic colony-forming cells, as well as antigen presentation by the immune system. IL-15, a four-alpha-helix bundle cytokine, stimulates activated T-cell proliferation and differentiation to effector T-cell subpopulations upon antigen-mediated activation. Moreover, IL-15 increases the maintenance of a memory phenotype of CD8+ CD44hi T cells [5]. IFNα is one of the type I interferons that activates the JAK-STAT signaling pathway, stimulating the activity of JAK1 and TYK2 proteins, leading to tyrosine phosphorylation of STAT1 and STAT2, and eventually inducing IL-4 secretion and B-cell activation. The toxicity of IFNα is usually dose-dependent, and the majority of the side effects can be managed without discontinuing treatment [3].

Through *in vivo* screening for anticancer effects, mRNA mixtures encoding four cytokines (IL-12sc, GM-CSF, IFNα, and IL-15 sushi) showed dramatic anticancer effects. Repeated intra-tumoral injections of the cytokine mRNA mixtures resulted in effective tumor growth control. Each cytokine was expressed within the tumor, and the serum cytokine concentration increased accordingly,

probably because of the cytokine secretion from the tumor to the TME. Moreover, most of the mice treated with a mixture of 4 cytokine mRNAs exhibited tumor regression, whereas mice given a single mRNA did not [11]. Thus, further *in vitro*, *in vivo*, and clinical studies are required to optimize the recipe of cytokine mRNA mixtures containing GM-CSF, IL15 sushi, IFNα, and scIL-12, as well as to verify the safety and efficiency of the antitumor activity with different tumor models.

4. Conclusion

The efficacy of mRNA cytokines for tumors can be improved by encapsulating mRNAs encoding cytokines with nanoparticles and by using mRNA mixtures encoding two or more synergistic cytokines-LNPs. In this review, three mRNA mixtures of cytokines, IL-12 and IL-27; IL-23, IL-36, and OX40L; GM-CSF, IL15 sushi, IFNα, and scIL-12, are highlighted. Better efficacy has been demonstrated by mixing two or more synergistic mRNA cytokines compared to applying one cytokine alone. Careful consideration and research on cytokine mRNA mixtures may facilitate the further development and clinical application of mRNA technology in cancer therapy and make tremendous advances in anticancer approaches.

References

[1]Mitra S, Das R, Emran TB, et al. Diallyl Disulfide: A Bioactive Garlic Compound with Anticancer Potential. Frontiers in Pharmacology, 2022, 13: 943967.

[2]Huang X, Kon E, Han X, et al. Nanotechnology-based strategies against SARS-CoV-2 variants. Nature Nanotechnology, 2022, 17(10): 1027-1037.

[3]Briukhovetska D, Dörr J, Endres S, et al. Interleukins in cancer: from biology to therapy. Nature Reviews Cancer, 2021, 21(8): 481-499.

[4]Liu C, Shi Q, Huang X, et al. mRNA-based cancer therapeutics. Nature Reviews Cancer, 2023, 23(8): 526-543.

[5]Waldmann TA. Cytokines in Cancer Immunotherapy. Cold Spring Harbor Perspectives in Biology. 2018, 10(12): a028472.

[6]Liu JQ, Zhang C, Zhang X, et al. Intratumoral delivery of IL-12 and IL-27 mRNA using lipid nanoparticles for cancer immunotherapy. Journal of Controlled Release, 2022, 345: 306-313.

[7]Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nature Reviews Immunology, 2003, 3(2): 133-46.

[8]Kaiga T, Sato M, Kaneda H, et al. Systemic administration of IL-23 induces potent antitumor immunity primarily mediated through Th1-type response in association with the endogenously expressed IL-12. Journal of Immunology, 2007, 178(12): 7571-80.

[9]Vigne S, Palmer G, Lamacchia C, et al. IL-36R ligands are potent regulators of dendritic and T cells. Blood, 2011, 118(22): 5813-23.

[10]Hewitt SL, Bai A, Bailey D, et al. Durable anticancer immunity from intratumoral administration of IL-23, IL-36γ, and OX40L mRNAs. Science Translational Medicine, 2019, 11(477): eaat9143.

[11]Hotz C, Wagenaar TR, Gieseke F, et al. Local delivery of mRNA-encoded cytokines promotes antitumor immunity and tumor eradication across multiple preclinical tumor models. Science Translational Medicine, 2021, 13(610): eabc7804.