Background Interleukin-12 (IL-12) is a potent immunotherapeutic cytokine in mouse models which application as a systemic agent in the clinical setting is hampered by IFN-γ-dependent toxicity.1 Engineering T cells with IL-12 is highly efficacious in mouse models2 but has resulted in serious adverse events in clinical settings.3 On the other hand, IL-18 is a myeloid-derived cytokine that elicits IFN-γ expression on T and NK lymphocytes.4

IL-12 and IL-18 are known to synergize in terms of eliciting massive IFNγ production.5 For cancer immunotherapy, IL-12 has the caveat of being down-regulated in its function by a decoy receptor termed IL-18BP,6 which is reportedly abundant in tumor tissues.7,8 Recently, a mutant sequence of mouse IL-18 termed DRIL18 which preserves its bioactivity but lacks binding to IL-18BP has been reported to exert T-cell-dependent antitumor activity upon systemic delivery.7

We previously reported that transient engineering of tumor-specific CD8 T cells with IL-12 mRNA enhanced their systemic therapeutic efficacy when delivered intratumorally.9 In this study, we sought to improve the therapeutic strategy of intratumoral delivery of T cells transiently engineered to express IL-12 with IL-18 mRNA electroporation.

Methods We mixed CD8+ T cells (TCR transgenic, TILs and CAR-T) engineered with mRNAs to transiently express either single-chain IL-12 (scIL-12) or an IL-18 decoy-resistant variant (DRIL18) that is not functionally hampered by IL-18BP. CD8+ T cells were injected repeatedly into mouse tumors for antitumor efficacy experiments. RNA-seq was performed to assess the functional changes induced after mRNA electroporation. Additionally, T-cell metabolic modifications and glycosylation profile functional changes were analyzed using Seahorse and cell adhesion assays.

Results Pmel-1 TCR-transgenic T cells electroporated with scIL-12 or DRIL18 mRNAs exerted powerful therapeutic effects in local and distant melanoma lesions. These effects were associated with T-cell metabolic fitness, enhanced miR-155 control of immunosuppressive target genes, enhanced expression of various cytokines and unique changes in the glycosylation profile of surface proteins, enabling enhanced adhesiveness to E-selectin. Efficacy of this intratumoral immunotherapeutic strategy was recapitulated using other clinically relevant adoptive T cell therapies as tumor-infiltrating lymphocytes (TILs) and CAR T cells upon IL-12 and DRIL18 mRNA electroporation.

Conclusions We report on a substantial improvement of adoptive T-cell therapies strategy based on mRNA transient gene-transfer and repeated intratumoral delivery. The synergistic immunobiology of IL-12 and IL-18, best represented in the form of DRIL18, holds promise for efficacious outcomes in the treatment of metastatic cancer patients.

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