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Targeted exosomes for co-delivery of siFGL1 and siTGF-β1 trigger combined cancer immunotherapy by remodeling immunosuppressive tumor microenvironment

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ABSTRACT

Immune checkpoint therapy encounters significant challenges in clinic, including low response rate, acquired resistance and immune-related adverse events. Combination immunotherapy targeting multiple independent but complementary pathways in immune evasion has the potential to enhance therapeutic efficacy. Herein, a combination therapeutic strategy that dually inhibiting FGL1, a recently discovered main ligand for immune checkpoint LAG-3, and TGF-β1, an immunosuppressive cytokine, is firstly reported for colorectal cancer immunotherapy by blocking immune checkpoint and modulating tumor microenvironment simultaneously. We established a cRGD-modified exosome with high siFGL1 and siTGF-β1 loading efficiency (cRGD-Exo/siMix) to realize the co-silence of FGL1 and TGF-β1. The constructed cRGD-Exo/siMix showed a significant anti-tumor effect both *in vitro* and *in vivo*. Analysis of the tumor immune microenvironment demonstrated an increased number of tumor infiltration $CD8^+$ T cells while a decreased number of immunosuppressive cells, implying that this therapeutic approach boosted anti-tumor immunity by reshaping the tumor microenvironment. This work provides a new strategy for siRNA delivery and its applications in combined cancer immunotherapy.

1. Introduction

Emerging immune checkpoint inhibitors (ICIs) show therapeutic potential in various malignant tumors with manageable toxicity and durable efficacy. However, only a certain portion of patients (usually below 20%) respond to this strategy even in susceptible cancers [1]. To increase the response rate, many efforts have been made. On one hand, new mechanisms for tumor immune evasion are constantly explored. For instance, Chen, *et. al* [2] recently reported that fibrinogen-like protein 1 (FGL1) was the main inhibitory ligand of lymphocyte activation gene-3 (LAG-3), which is considered to be the primary target other than PD-1 in the development of cancer therapy [3]. On the other hand, combination immunotherapy has been attempted in clinical trials [4,5]. Recent trials reported that compared with monotherapy or chemotherapy, the combination of ICIs achieves better outcomes in many cancer types [6,7]. Besides, dual inhibition of immune checkpoint and target related to the tumor microenvironment (TME) is another plausible therapeutic strategy because they are two independent and complementary pathways in the process of immune evasion [8,9].

Transforming growth factor-β (TGF-β) is an immunosuppressive cytokine in TME, which can promote tumor progression and mediate immune escape by recruiting immunosuppressive cells and forming hard matrix [10,11]. Blocking TGF-β in TME is expected to achieve the transition from a cold tumor to a hot tumor by promoting the infiltration of immune cells [12]. In recent trials, a bifunctional fusion protein that simultaneously blocking PD-L1 and TGF-β has been applied to patients with different types of advanced solid tumors, resulting in impressively durable responses [13–15]. Despite the durable benefits of current combination immunotherapy attempts in clinical trials, the limitations related to these regimens must be considered. First, antibody immune

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