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Targeted delivery of let-7b to reprogramme tumor-associated macrophages and tumor infiltrating dendritic cells for tumor rejection

Zhen Huang [a, 1], Jingjing Gan [a, 1], Ziyan Long [a], Guangxing Guo [a], Xiafei Shi [a], Chunming Wang [b], Yuhui Zang [a], Zhi Ding [a], Jiangning Chen [a], Junfeng Zhang [a, **], Lei Dong [a, *]

[a] State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing, 210093, China
[b] State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macau SAR, China

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ABSTRACT

Both tumor associated macrophages (TAMs) and tumor infiltrating dendritic cells (TIDCs) are important components in the tumor microenvironment that mediate tumor immunosuppression and promote cancer progression. Targeting these cells and altering their phenotypes may become a new strategy to recover their anti-tumor activities and thereby restore the local immune surveillance against tumor. In this study, we constructed a nucleic acid delivery system for the delivery of let-7b, a synthetic microRNA mimic. Our carrier has an affinity for the mannose receptors on TAMs/TIDCs and is responsive to the low-pH tumor microenvironment. The delivery of let-7b could reactivate TAMs/TIDCs by acting as a TLR-7 agonist and suppressing IL-10 production *in vitro*. In a breast cancer mouse model, let-7b delivered by this system efficiently reprogrammed the functions of TAMs/TIDCs, reversed the suppressive tumor microenvironment, and inhibited tumor growth. Taken together, this strategy, designed based upon TAMs/TIDCs-targeting delivery and the dual biological functions of let-7b (TLR-7 ligand and IL-10 inhibitor), may provide a new approach for cancer immunotherapy.

1. Introduction

Cancer immunotherapy aims to harness the power of both the innate and adaptive immunity to eliminate tumor cells [1]. As one of the most promising cancer treatment approaches, its therapeutic efficacy may be greatly impaired by the immunosuppressive tumor microenvironment [2]. In this environment reside the infiltrating immune cells, including tumor-associated macrophages (TAMs) and tumor infiltrating dendritic cells (TIDCs), which play essential roles in orchestrating tumor immunosuppression. Specifically, TAMs with an IL-12^{low}IL-10^{high} phenotype suppress T cell activation and proliferation, while promoting angiogenesis by secreting IL-10, TGF-β and a large amount of pro-angiogenic factors [3]. Meanwhile, TIDCs are functionally deficient, unable to adequately induce an adaptive immune response to tumor antigens. They lose the ability to secrete pro-inflammatory cytokine such as IL-12 and even support the expansion of Treg cells [4]. Therefore, both TAMs and TIDCs have become promising therapeutic targets for breaking down the tumor environment. Several immunostimulatory reagents have been tested on both TAMs and TIDCs *in vitro* [5]. However, because TAM usually out numbers DC in the tumor and TIDC keep moving between the tumor tissue and the draining lymph node, it is difficult to confirm these drugs, aimed at targeting TAMs by design, could also effectively activate TIDCs at the same time *in vivo* [6,7]. In consequence, it is highly desirable to design a rational administration method and select optimized drugs to target both TAMs and TIDCs in the initial stage, which may further improve the therapeutic effect of immunotherapy.

Meanwhile, due to the refractory property of TAMs and TIDCs, different types of therapeutic reagents, such as TLR ligands, antibodies against immunosuppressive cytokines and immunostimulating cytokines, have been combined to reactivate these myeloid cells [8,9]. But these reagents are usually systematically administered and may induce uncontrolled inflammation throughout the body [10]. Moreover, the interaction between these drugs use together may cause uncertain effects in clinical treatment [11]. Thus, a desirable therapeutic candidate should ideally possess the

* Corresponding author.
** Corresponding author.
E-mail addresses: jfzhang@nju.edu.cn (J. Zhang), leidong@nju.edu.cn (L. Dong).
[1] The two authors contributed equally to this work.

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functions to reactivate these cells while being delivered in an appropriate way to target TAMs and TIDCs. As a microRNA member, let-7b can suppress IL-10 production in T cells and activate microglia by acting as the ligand of TLR-7 [12,13]. IL-10 is one of the most potent immunoregulatory cytokines responsible for the intratumor immunological tolerance, whereas other TLR-7 agonists have been proved to improve the DC-based immunotherapy against melanoma [14,15]. To fulfil the therapeutic potential of let-7b, a rationally designed nucleic acid vector is in great demand for the delivery of this microRNA mimic to target TAMs and TIDCs in the tumor microenvironment.

In this study, a nucleic acid drug delivery system targeting both TAMs and TIDCs was constructed and tested for its potential in cancer immunotherapy. Due to the overlapping expression of mannose receptor (MR) on TAMs and TIDCs, cationic *Bletilla Striata* polysaccharide (cBSP), which contains high mannose moieties, was used to conjugate let-7b to form the nano-complex [16]. Then, a previously established pH-responsive material, PEG-histamine-modified alginate (PHA), could combine with cBSP & let-7b to form the multi-component complex which can specifically release the cBSP & let-7b in response to the tumor acidic microenvironment [17]. The schematic diagram of the co-targeting delivery system is shown in Fig. 1. We examined the ability of the multi-component system for targeting TAMs and TIDCs and evaluated its therapeutic effect in a mouse model of breast cancer.

2. Materials and methods

2.1. Reagents and synthesis of materials

The let-7b (5′- UGAGGUAGUAGGUUGUGUGGUU-3′) and mutant oligonucleotide (mut. oligo, 5′-UGAGGUAGAAGGAUAUAAGGAU-3′), antisense oligonucleotide (ASO) against IL-10 (5′-ATGCGCGA-TACGCGTAC-3′) with all nucleotide with phosphorothioate modification were synthesized by Life Technology (La Jolla, CA, USA). Chloroquine and ssRNA40/LyoVec™ was purchased from InvivoGen (Hong Kong, China). For cellular localization and tissue distribution, cyanine 3-labeled let-7b (Cy3-let-7b, for *in vitro* transfection) and Cy5.5-labeled let-7b (for *in vivo* tissue distribution) were applied. BSP was isolated and purified according to our previous report [16]. Other chemical agents and enzymes were obtained from Sangon Biotech (Shanghai, China).

Cationic BSP (cBSP) was prepared by incorporating ethylenediamine within the hydroxyl groups of BSP via an *N*, *N*′-carbonyldiimidazole (CDI) activation protocol with the mole ratios between CDI and the hydroxy group of BSP ([CDI] [OH] = 3), as previously report [16]. PEG-His-modified alginate (PHA) was also prepared as previous described [17]. cBSP&let-7b complex was formed by mixing a volume of 5 mg/ml of let-7bwater solution with 3 vol of 5 mg/ml of cBSP saline solution. Then, the formed cBSP&let-7b complex solution and 5 mg/ml PHA solution was mixed at the volume ration of 1:1 via gentle pipetting to form the triple complex (named as let-7b & vector).

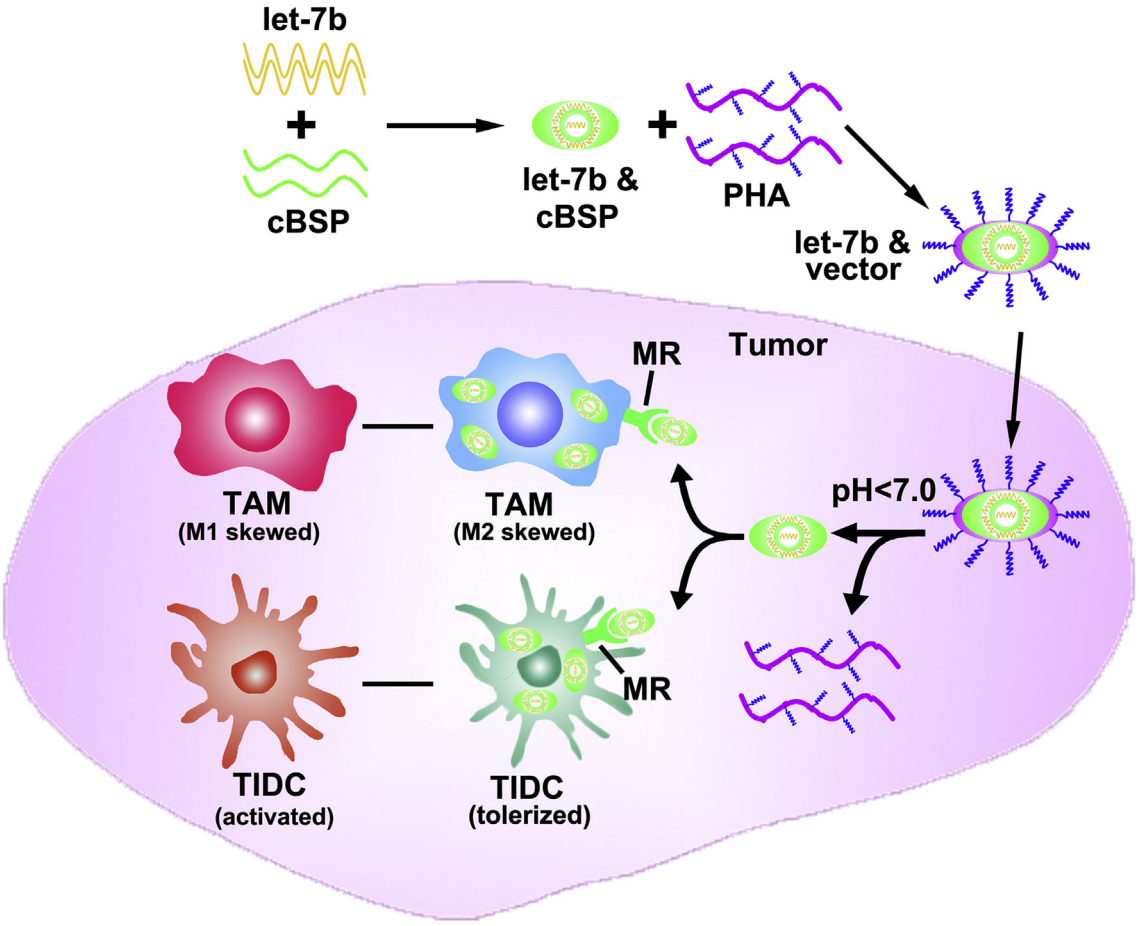

Fig. 1. The schematic diagram of the TAMs and TIDCs co-targeted nucleic acid drug delivery system. TAM, tumor-associated macrophage; TIDC, tumor infiltrating dendritic cell; cBSP, cationic *Bletilla Striata* polysaccharide; PHA, PEG-histamine-modified alginate; MR, mannose receptor.