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Modified YSK12-MEND-siRNA in Dendritic Cells for Cancer Immunotherapy

Syed S. Alam
Virginia Commonwealth University

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Shaheer Alam, Margaret Tinsley
Biology, Virginia Commonwealth University

Abstract

Tumors may induce tolerogenesis through signaling dendritic cells to produce tolerogenic molecules, such as indoleamine 2, 3-dioxygenase 1 (IDO1). Tumor-associated immunosuppression is associated with higher mortality in patients. Small interfering RNA (siRNA) has been shown to silence specific target genes in the target cell. The siRNA associated with these genes could support a gene knockdown of these immunosuppressors and reduce mortality. Delivery of these therapeutic nucleic acids is difficult *in vivo* because siRNA is easily broken down inside the cell and the bloodstream through present nucleases. Use of liposome polymers has been reviewed extensively in literature. YSK12-C4, a lipid nanoparticle developed by Hokkaido University, is a lipid that has both fusogenic and cationic properties, making it ideal for dendritic cell uptake. However, limitations make it less effective *in vivo* as it may collect in areas other than the target human dendritic cells in the draining lymph node. To improve specific targeting *in vivo*, ligand-based targeting modifications (anti-DEC205, anti-CD11c, mannose+) and physical targeting (pKa modifications through YSK05 lipid addition) have been proposed to the YSK12-MEND system in order to better target dendritic cells *in vivo*. In order to estimate the theoretical efficacy of modified YSK12-MEND-siRNA, I investigated prior experiments, both *in vivo* and *in vitro*, to recognize the practicality of each modification. In murine hepatocytes, it was found that pKa improved specific uptake *in vivo* while anti-DEC205 and anti-CD11c peptides were found to be effective in dendritic cells *in vivo* in mice. Mannose+ lipid nanoparticles were ideal for targeting both dendritic cells and macrophages. These modifications will likely result in an improved uptake, more cell specificity, and transfection efficiency for human dendritic cells.

Keywords: YSK12-C4, dendritic cells, mannose, pKa, DEC205, CD11c, specific targeting

Introduction

Dendritic cells (DCs) are antigen presenting cells, which through phagocytosis, intake particulates for processing, and presenting to lymphocytes, namely CD4+ T cells and CD8+ T cells.[1] Some tumors are capable of inhibiting normal immune function by releasing tumor-associated immunosuppressors, immunomodulating dendritic cells to signal to T cells through the T-cell receptor to enter cell cycle arrest or apoptosis.[2] Using siRNA to prevent the dendritic cells from producing these immunosuppressors, cancer mortality can decrease. Methods on deliviering siRNA require improvement. A cationic liposome, YSK12, is promising but could be significantly improved so that dendritic cells can be specifically targeted in an efficient manner. Ligand-based targeting systems and physical targeting systems are of key interest for direct human intake of nucleic acid therapeutics.[3]

Methodology

This project was done for HONR 200 Rhetoric, a class designed for reviewing literature for the purpose of finding a given conclusion. This project is made up of a review of different sources for the investigation of potential modifications.

Results

- YSK12-MEND was found to have a zeta potential of 5.8 mV and ~160 nm in particle size[2]
- The YSK12-MEND alone had 70% silencing of indoleamine 2, 3-dioxygenase 1 (IDO1) at dose of 20 nM in murine bone marrow-derived dendritic cells (BMDCs)[4]
  - Tumor growth reduced by ~60% over 20 day period for both IDO1 and suppressor of cytokine signaling 1 (SOCS1)[2, 4]
- Encapsulation of siRNA was 87-90%[4]
- pKa of YSK12-MEND was 8.00[5]
  - Can be adjusted by adding with secondary lipid
  - pKa lowered to 7.15 with addition of YSK05 in a 5:2 ratio
    - Unequal contribution from each lipid
- 0.6 mg/kg dose using anti-DEC205 peptides resulted in >50% of CD11c+CD8a+ cells uptaking the lipid nanoparticle[6]
  - 75% reduction in CD80 in affected DCs
- P-D2, derived from intercellular adhesion molecule 4 (ICAM4), demonstrated >50% uptake in DCs[7]
  - Adheres to the CD11c protein on DCs

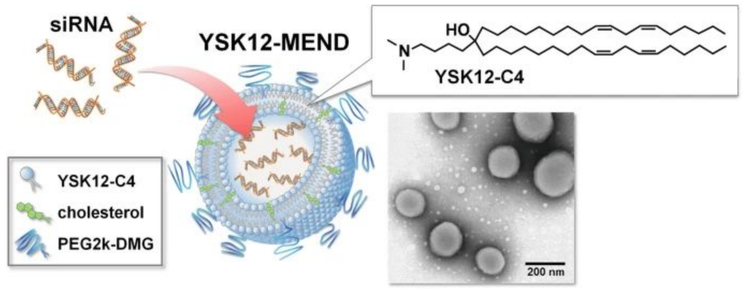

Figure 1. The composition of YSK12-MEND. The MEND system allows at least two ligands to attach to it for specific use. Figure adapted from Khalil et al., 2019.

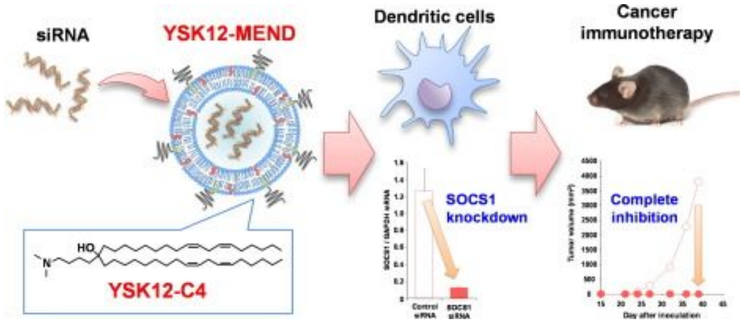

Figure 2. Introduction of siRNA through YSK12-MEND. The siRNA enters the DC after the initial phagocytosis and endosome disruption. mRNA for SOCS1 is inhibited through RISC slicing. Figure adapted from Warashina et al., 2016.

Conclusion

YSK12-MEND was found to be an efficient transfection reagent in isolated BMDCs and were relatively efficient in live mice with individual modifications. In the studies reviewed, other peptides or antibodies for certain receptors were identified but did not yield significant results to be considered for YSK12-MEND. The increased uptake and cell specificity from pKa modification, antiDEC205 peptides, and antiCD11c peptides show promise for maximizing the *in vivo* application of YSK12-MEND, ideally for cancer immunotherapy. While only studies applying siRNA were reviewed, miRNA, mRNA, or pDNA, etc. treatments are also viable within this transfection reagent, although further study is required for the interactions between the liposome and these nucleic acids.

While cationic liposomes are generally considered ideal for transfection for DCs, new findings suggest that these positively charged particles merely adhere to the cell surface and are not taken in, whereas anionic or neutral liposomes may function better.[9] YSK12-C4 is a cationic lipid but the MEND system could be made anionic through other ligands. Further investigation into YSK12-MEND using imaging flow cytometry is needed.

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