Functional delivery of miR-218 precursor in primary bronchial epithelial cells using 3DNA® Nanotechnology

Introduction

MicroRNAs are small non-coding RNAs 21-23 bases long that function in a post-transcription regulatory role to control the translation of mRNA into protein. (1) These small RNAs have essential roles in development and regulation of cellular timing, and have been implicated in cancer and infectious disease progression (1,2). The biogenesis of miRNAs begins with transcription of a long precursor RNA, pri-microRNA, in the nucleus. Pri-microRNAs are further process by nuclear RNases into short hairpin molecules, pre-microRNAs. Pre-microRNA are actively transported into the cytoplasm where they are cleaved by the enzyme Dicer into mature miRNA species and integrated into RNA-Induced Silencing Complexes (1). Recently pre-miRNAs, like siRNAs, have been used independently as therapeutic agents to control cellular processes, and in combination with other therapeutics to improve therapeutic efficacy (2). While the search for the ideal delivery system for small RNA molecules (siRNA and miRNA) continues to progress slowly, nanotechnology as a therapeutic delivery solution provides many new options for novel delivery constructs. One such complex, the 3DNA® nanocarrier, consists of a core of double stranded DNA with hundreds of single stranded peripheral sites for the attachment of therapeutics and targeting agents. An example of a 3DNA® with antibody targeting agent and drug cargo is shown in Figure 1.

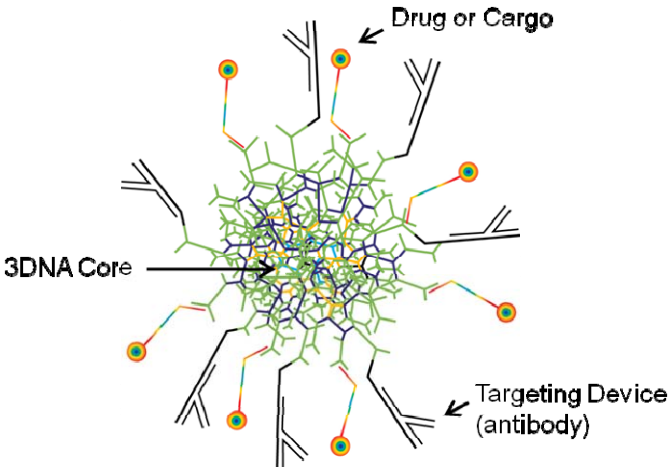


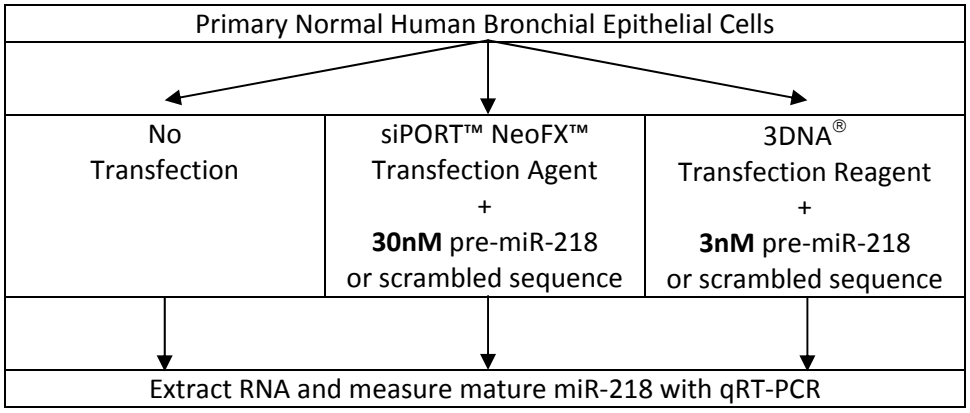
Figure 1: 3DNA® nanocarrier

In previous work, Dr. Silvia Muro at University of Pennsylvania (currently at University of Maryland College Park) was the first to demonstrate 3DNA®-dependent cellular targeting, internalization, and endosomal escape (3). We combined the targeting and delivery capabilities of 3DNA® nanoparticles with the therapeutic potential of microRNAs to deliver pre-miR-218 into primary bronchial epithelial cells based on work done in the Spira lab at Boston University (4).

Methods and Materials

Custom 3DNA® reagents. Since epithelial cells express surface intercellular adhesion molecule-1 (ICAM-1), 3DNA® nanoparticles were custom manufactured with multiple anti-ICAM-1 antibodies per 3DNA®, as groups of anti-ICAM-1s have been shown to assist cellular internalization (3,5).

Cellular Transfection, RNA Isolation and qRT-PCR. Primary normal human bronchial epithelial (NHBE) cells were purchased from Cell Applications, plated at 30% confluence in 6-well plates, and cultured in optimized growth media (Cell Applications). NHBE cells (6 replicates per condition) were transfected with pre-miR-218 or a negative control scrambled sequence (Ambion/Life Technologies), using ICAM-1 targeted 3DNA® nanoparticle or siPORT™ NeoFX™ Transfection Agent (Ambion/Life Technologies). Parallel no-transfection conditions were also run:



Cells were harvested 48 hours after transfection and RNA was isolated using the miRNeasy mini kit (Qiagen) to obtain Low Molecular Weight RNA. Ten nanograms of each RNA was used in triplicate TaqMan® qRT-PCR assays (Applied Biosystems) for miR-218, U6, and other microRNAs. Data from all qRT-PCR experiments were normalized to U6 and analyzed using the comparative Ct method (6).

Results

In order to demonstrate functional delivery of a cargo molecule into a primary cell line, we chose to study pre-miRNA transfection of miR-218 and its conversion to mature miRNA in the cytoplasm. Since only mature miRNA, not pre-miRNA, is detected by TaqMan® qRT-PCR, we used this distinguishing capability as a means to confirm transport into the cell, release into the cytoplasm, and conversion into mature product. As illustrated in Figure 2, pre-miR-218 was combined with 3DNA® targeted to cell-surface ICAM-1, and incubated with primary cells in culture to passively deliver pre-miR-218. In parallel, we used siPORT™ NeoFX™ Transfection Agent for comparison and to confirm the pre-miR-218 and scrambled oligos were functioning properly.

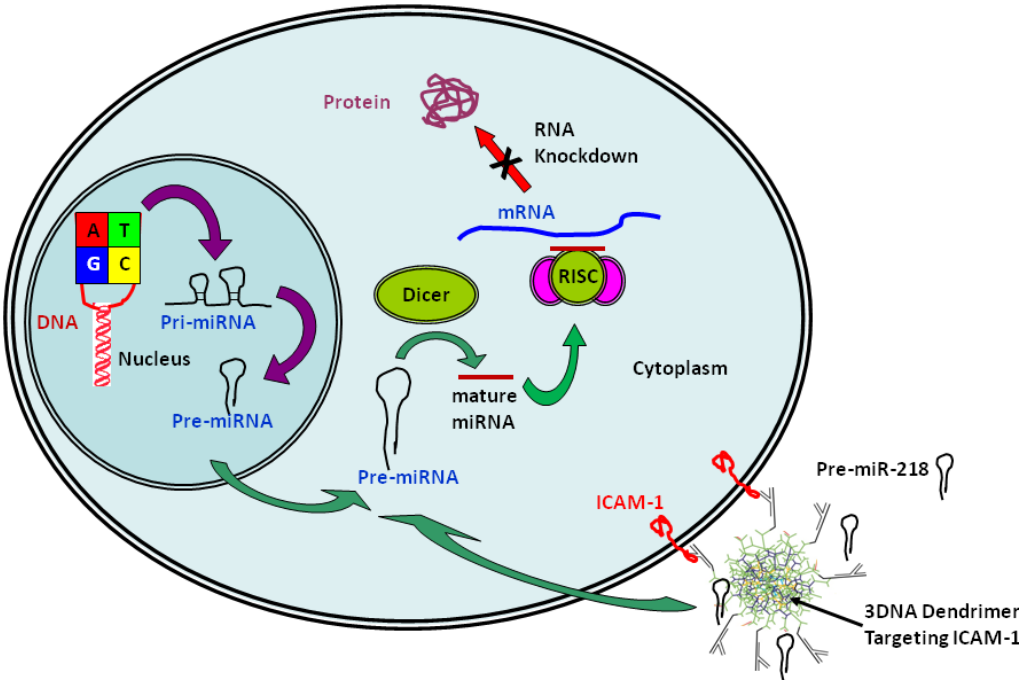


Figure 2: 3DNA® Transfection Overview

Both transfection reagents resulted in an effective cellular uptake of the precursor and enzymatic conversion into the mature form, compared to non-transfected controls. Relative expression patterns of miR-218 in each of the experimental conditions are plotted in Figure 3. As expected, the scrambled pre-miR sequence demonstrated little or no induction of mature miR-218 (similar to non-transfected controls).

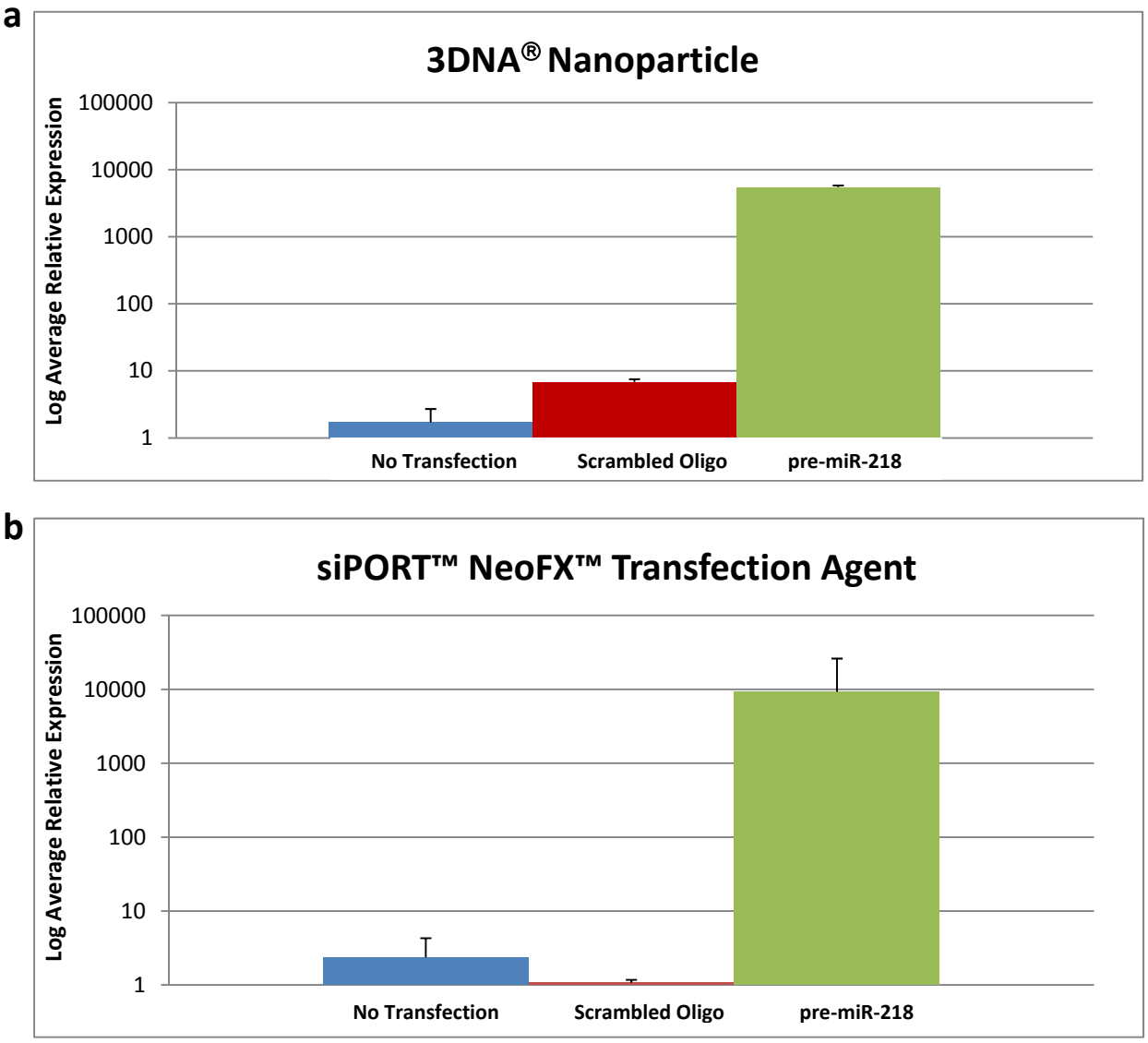


Figure 3: Transfection Results

Interestingly, similar expression of miR-218 was observed in 3DNA®-transfected and siPORT™ NeoFx™-transfected cells (Figures 3a and 3b), even though the concentration of pre-miR-218 oligo was 10 times less in 3DNA®-transfected cells (3nM for 3DNA® vs 30nM for siPORT™ NeoFx™). This finding suggests 3DNA® nanocarriers enhance cellular uptake and/or endosomal release of the pre-miR-218.

References

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Acknowledgement

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