Transfection of Prostate PC3 cells with Metafectene

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Materials
Plasmid DNA to inhibit CD44v9 infection or Muc18 infection
Metafectene or Lipid L
RPMI medium without serum and antibiotics.

A comparison of Metafectene with Lipid L in prostate PC3 cells has not been performed, and this is worth pursuing in order to determine its potential for gene therapy. We have evidence for increased cell adhesion molecule CD44 variant 7-10 expression in prostate cancer compared to benign prostate, and hypothesized that abrogation of this expression could reduce the metastatic potential of the cancer. As a prerequisite to nonviral gene therapy, we evaluated the possibility of transfecting confluent aggressive prostate cancer cell line Gs-alpha (This is a PC3 cell line that overexpresses the Gs-alpha protein and which constitutively activates its Gs-alpha signal transduction protein) by Metafectene and compared the transfection efficiency, cell viability, and expression of the gene of interest with that achieved by Lipid L.

We employed RNAi to study the functions of CD44 variant 9 (v9), whose expression we have shown to be increased at the mRNA and protein levels in prostate cancer cells, and a cell adhesion marker studied by others in prostate cancer, called Muc18. The sequences of both were found in GenBank. The strategy used in this study was to design and synthesize two pairs of 21 bp oligonucleotides for each molecule (two sense and two antisense) based on a 21-DNA nucleotide fragment of each molecule. Preparation of the RNAi construct involved annealing complementary oligonucleotides mixing and boiling them, cooling them to anneal, and cloning them into plasmid vector U6pBS. The goal in transfection experiments was to have the cell use the U6 promoter to express short hairpin RNAs in transfected cells, and specifically suppress gene activity.

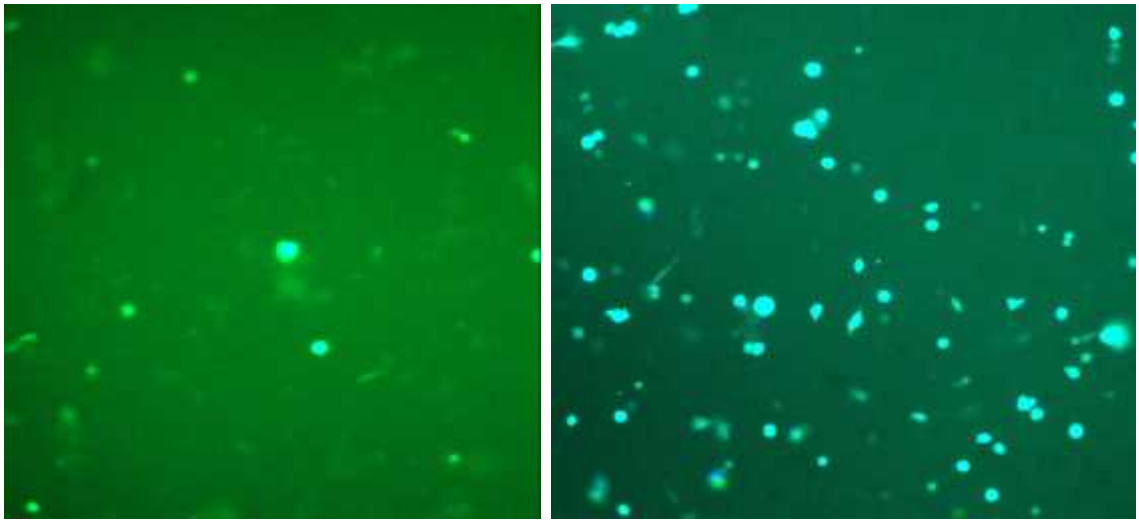
Transfection of Gs-alpha cells with vector U6pBS having RNAi constructs was performed using Metafectene (Biontex) and Lipid L separately. In brief, cells were plated at a density of $5x10^5$ cells per well in 2 ml of RPMI medium with serum and antibiotics in 6-well plate. Cells were then transfected 24 hr later with vector U6pBS containing either RNAi construct:Metafectene complexes or RNAi construct: Lipid L complexes. To formulate RNAi construct:lipofection complexes, two tubes were filled with 100 µL of RPMI without serum or antibiotics. To one tube, 10 µL (10 µg) of PCI-neo-GFP plasmid (which produces green fluorescent protein) as a reporter was added, followed by 4 µL (4 µg) of RNAi construct (for CD44v9 or Muc18). To the second tube, 10 µL of Metafectene or 25µL of Lipid L (maximum amount recommended by the manufacturer) were added. The contents of the two tubes were mixed well, then allowed to stand for 25 min to allow formation of lipofection agent:DNA complexes for transfection. The old serum-containing medium was removed and fresh 800 µL of serum-containing medium was added to each well receiving Metafectene. In the wells receiving Lipid L, the old serum-containing medium was also removed and the wells were rinsed with 1xPBS before adding 800 µL RPMI without serum and antibiotics. The incubated complex was then added dropwise to the cells in each well and plates were agitated by slow movement to distribute the transfection complexes. Plates were incubated for 6 hours in a 5% CO_2 incubator. The transfected cells were rescued by removing the transfection medium and adding 5 ml of regular medium and incubating for 90 hours more, as we have found necessary to deplete the protein translated while RNAi construct abrogate the expression of CD44 gene, then cells were harvested for use in Matrigel invasion assay and Western blot analysis Cell counts were performed using Trypan blue dye exclusion after this time. In five separate experiments, after 6 hours of transfection, Metafectene-

transfected cells consistently showed much greater transfection efficiency than did Lipid L -treated cells (Figure 1A-B). Cell counts using Trypan blue dye exclusion revealed a cell viability of 68% to 81% in the Lipid L -treated cells and 73% to 83% in the Metafectene-treated cells.

Conclusions

We confirm that Metafectene is more efficient than Lipid L in transfecting cultured cells. The use of 40% (10 µL versus 25 µL) as much Metafectene was much more effective in transforming cells. The cell survival was comparable with each treatment.

The working dogma says that the majority of cells transfected with GFP are successfully co-transfected with the another oligonucleotide, in this case the RNAi construct. The ratio between the construct and GFP was skewed at 4:10. Thus, it can be assumed that the green cells represent the entire population of cells which carry the construct in our experiments. More Metafectene-transfected cells than Lipid L-transfected cells should carry the desired construct, and so separation of the GFP-positive cells by flow cytometry will provide more cells for Matrigel invasion studies.



A **B**

Legends for Figures

Figure 1. Comparative transfection efficiency of 25 µL Lipid L and 10 µL Metafectene, with co-transfection of green fluorescent protein (GFP) as a reporter. A. Confluent Gs-alpha cells after Lipid L-mediated transfection for MUC18 interference with few cells showing GFP signal (100x). B. Similarly confluent cells after Metafectene-mediated transfection for MUC18 interference. More cells, about two dozen, consistently showed GFP signal (100x).

Acknowlegements

Plasmid U6pBS and PCI-neo-GFP plasmid were gifts from Dr. Steven Sugrue, Department of Anatomy and Cell Biology, University of Florida. Prostate cancer cell line Gs-alpha was a gift from Dr. Girish Shah, Department of Pharmacology, University of Louisiana, Monroe, LA.