260b Quantitative Evaluation of the Role of Vector in the Dynamics of Antisense Effects

Sumati Sundaram, Li Kim Lee, and Charles Roth

Antisense technology employs short, single-stranded oligonucleotides (ODNs) to inhibit gene expression by binding to complementary mRNA via Watson-Crick base pairing. However, poor cellular delivery of the ODNs impedes their widespread utilization in therapy and biotechnological research. Numerous delivery agents have already been developed to enhance their cellular uptake while also protecting them from degradation. However, further improvements in the design of carriers for enhanced ODN delivery demand a better understanding of the role of the vector on the extent and time course of the antisense effect. Particularly, the amount of time spent by the ODN in the cellular milieu, prior to binding with the mRNA, will determine the extent of exposure of the ODNs to nucleases and the subsequent availability of ODNs for producing an antisense effect. Vector design properties play a key role in determining the intracellular fates of ODNs by influencing both the ability to escape lysosomal degradation pathways and also the kinetics and location of carrier/ODN complex dissociation for ODN release. Therefore, significant insights can be obtained by understanding the relationships between the dynamics of vector mediated ODN delivery and antisense inhibition levels. The objective of this work is to examine the role of the vector on this interplay.

We accomplish this by measuring simultaneously the dynamics of both ODN uptake and antisense inhibition using a cellular assay based on single cell fluorescence measurements. Typically, ODNs (an effective anti-pd1EGFP sequence and its modification with a 5' red fluorophore conjugation) are delivered using a model polymer polyethyleneimine (PEI) in stably expressing pd1EGFP CHO cells. Cells treated with PEI/ODN complexes are subsequently subjected to flow cytometry at various time points to analyze for red (uptake) and green (antisense activity) fluorescence. Further, we utilize two parameters -- the polymer molecular weight and the ODN chemistry -- to alter the strength of carrier/ODN association and the rates of ODN degradation. This will enable us to perturb the dynamics of intracellular ODN availability and observe its effect on the appearance of the antisense effect. Implications of these results for the rational design of ODN delivery vectors will be discussed.