222-C A DISRUPTIVE SET OF TOOLS TO REALIZE THE PROMISE OF THE GENOMICS REVOLUTION

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Background Effective delivery of antisense oligonucleotides (ASOs) and small interfering RNAs (siRNAs) has long been a coveted goal for successful genetic therapy. However, despite considerable effort, therapeutics in which transcription and/or translation of specific gene sequences is blocked remain largely aspirational due to the inefficiency of existing delivery technologies and/or unacceptable toxicity profiles of current delivery vehicles. We have developed and have now obtained proof of concept for a platform technology that overcomes the limitations of previous oligonucleotide delivery systems.

Methods The platform we have developed has been the foundation for peptide-based research reagents for measuring protease activities inside live cells, including single cell measurements following delivery of lethal hits to tumor targets by cytotoxic lymphocytes. Modification of the chemistry has now allowed highly efficient, nontoxic delivery of therapeutic oligonucleotides. Recent confirmatory physical measurements further define the structural basis of these probes allowing delivery therapeutic for ASOs and siRNAs into tumor cells and immunotherapeutic lymphoid cells.

Results Using a series of oligonucleotides with various specific as well as randomized sequences, delivery efficacy was found to be *nucleic acid sequence-independent*. This was followed by affirmation that modified oligonucleotide constructs were able to enter a variety of cell types including carcinomas and leukocytes without any detectable toxic effects. Of high therapeutic potential, expression of the messenger RNA (mRNA) level for a targeted gene was successfully knocked down (as measured by PCR) in the bone marrow, liver, spleen, smooth muscle, and striated muscle of transgenic mice following injection into mouse tail veins of an siRNA. Additionally, we have covalently modified monoclonal antibodies (mAbs) such that they are linked to protease substrates specific for proteases that enable metastasis; the latter are also covalently linked to oligonucleotides bearing this unique oligonucleotide delivery moiety. Cleavage of the protease substrates by the protease on the tumor cell surface results in localization of oligonucleotides at a targeted cell surface. Oligonucleotides (both single strand DNA and double strand RNA) are then able to diffuse into the tumor cell or lymphocyte and inhibit translation of targeted mRNAs.

Conclusions The above-described delivery system is a technology platform that is capable of highly efficient, nontoxic delivery of therapeutic and diagnostic oligonucleotides currently focusing on metastatic cancer cells and checkpoint protein expression on lymphoid cells.

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