Dynamic Polyconjugates (DPC) Technology: An Elegant Solution to the siRNA Delivery Problem

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RNAi as a Therapeutic Modality

Harnessing biological pathways to treat disease is a highly attractive and potentially powerful approach. Biological pathways are extraordinarily efficient in carrying out their function in the body; such efficiencies are rarely achieved using conventional medicines. One pathway that is currently being explored for therapeutic applications is RNA interference (RNAi). RNAi was discovered by Fire and Mello, for which they were awarded the Nobel Prize in Physiology or Medicine in 2006. The RNAi pathway is present in all cells of the human body and operates as a sequence-specific gene silencing mechanism triggered by the presence of double-stranded RNA (dsRNA). In 2001, Tuschl discovered that short versions of dsRNA, known as small interfering RNAs (siRNAs), could be used to trigger RNAi. siRNA, and its analogsare presently being evaluated as drugs to target genes associated with disease. With the sequence of the human genome in hand and our increased understanding of the molecular basis of disease, RNAi is poised to be a transformative technology in the pharmaceutical industry.

Barriers exist to the use of siRNAs as a drug. Naked siRNA has poor pharmacological properties after systemic administration, the required route for most disease applications, including poor circulation times in the bloodstream and rapidly clearance from the body, leaving very little siRNA available for uptake into target tissues. The small amount of siRNA that does reach the target tissue is taken up by cells into membrane bound degradative compartments such as endosomeswhere it is prevented from accessing the cell's RNAi machinery in the cytoplasm and is rapidly degraded. Entrapment of siRNA in these compartments has frustrated many systemic siRNA delivery approaches. A technology that is able to overcome these barriers and enable safe and effective siRNA delivery remains highly sought after.

Dynamic Polyconjugates – An Elegant Solution to the siRNA Delivery Problem

Dynamic Polyconjugate (DPC) technology is engineered to overcome the barriers to systemic administration of siRNA. First developed by Arrowhead Madison scientists in 2007, the

inspiration for DPC technology came from the physical characteristics of viruses, nature's own nanoparticles for nucleic acid delivery. Viruses are efficient at finding their target cells and delivering their nucleic acid payload to the proper cellular compartment. Key features of viruses are their small size, their overall negative surface charge, their exquisite specificity for particular cell types based on receptors unique to that cell, and their ability to disassemble and release their nucleic acid cargo to the proper cell compartment in response to cellular triggers. All of these features are incorporated into DPC technology.

DPCs are small nanoparticles 5-20 nm in size and are composed of an amphipathic polymer to which shielding agents such as PEG, as well as targeting ligands and the siRNA are reversibly attached. The polymer is selected for its membrane lytic ability, which is dependent both on the positive charge of the amines on the polymer as well as its hydrophobic character. These amines are modified, or "masked", using proprietary maleic anhydride derivatives called "CDMs" to form acid-labile maleamate groups with a slightly negative charge.

Masking of the polymer's amines accomplishes two interrelated objectives that are critical to *in vivo* siRNA delivery:

- Reduction of toxicity by controlling when the membrane lytic property of the polymer is activated, and
- Inhibition non-specific interactions with blood components and non-targeted cell types.

The CDM derivatives used in DPC technology include those that contain PEG to enhance serum stability or a ligand to direct the DPC to a particular cell type. The CDM linkage on a polymeric amine is relatively stable at physiological pH, but is fully reversible in the acidic conditions encountered in the endosome. Unmasking of the polymer's amines in the endosome results in reactivation of its membrane lytic activity and endosomal disruption. Final DPC disassembly is achieved when the siRNA, attached to the polymer via a disulfide bond, is released from the polymer in response to the reducing environment of the cytoplasm. There it engages the cell's RNAi machinery, ultimately resulting in knockdown of target gene expression.

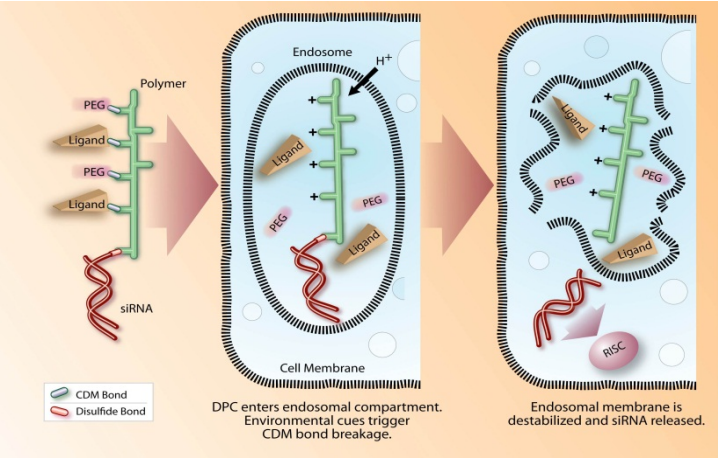

Figure 1: Schematic of the DPC system and mechanism of siRNA delivery

DPC technology is radically different from standard liposomal or lipid nanoparticle siRNA delivery systems used by the majority of RNAi therapeutics companies for systemic delivery of siRNA. First, DPCs are much smaller than lipid-based systems, enabling more efficient egress from the vasculature to access the target tissue. Second, DPCs can use targeting ligands for cell-type specific delivery, something that has yet to be achieved with the lipid-based systems used in clinical development programs. Third, the modular nature of DPCs allows each component to be optimized for the highest efficacy and lowest toxicity. As a polymer-based system, DPCs are fundamentally different from lipid-based systems. This opens up an entirely new class of macromolecules to enable siRNA delivery.

Recent Advances in DPC Technology

Since our initial publication describing DPC technology in 2007, our research group in Madison has worked to improve individual DPC components with respect to efficacy, safety and manufacturability. The prototypical DPC polymer was a polyvinylether named “PBAVE” that was produced by uncontrolled polymerization. The uncontrolled nature of the polymerization resulted in polymers that were heterogeneous in size and composition, necessitating the use of sophisticated purification methods and making reproducibility and analytics difficult. Using controlled radical polymerizations such as atom transfer radical polymerization (ATRP) and reversible addition-fragmentation chain transfer (RAFT), we are now able to produce polymers that are homogeneous and more amenable to large scale manufacturing. We currently possess several generations of RAFT polymers that are optimized and screened for specific delivery requirements. These include polymers containing hydrolysable bonds in both the main and side chains that may be repeatedly injected without concomitant increases in toxicity.

Another major advancement of DPC vehicles is the masking group, which is based upon the reversible modification of amines by maleic anhydrides to form acid-labile maleamate groups. The stability of original CDM masking chemistry in the bloodstream is effective for DPCs designed for hepatocyte delivery, but was not optimal for delivery to other tissues where access is not as easily acheived. In order to enable longer circulation times to enhance access to these tissues, we developed masking chemistry also based upon maleic anhydride but with maleamate groups that are more stable under physiological conditions. By systematic variation of the maleic anhydride substituents, we have created delivery vehicles with prolonged circulation. These facilitate delivery to other target tissues including tumors in which extravasation is relatively slow.

Targeting DPCs

An attractive feature of the DPC technology is the ability to target cells of interest via cell-type specific ligands. The prototypical DPC was designed to target liver hepatocytes by attaching N-acetyl-galactosamine (NAG) to the core DPC polymer. The NAG ligand has high affinity for the asialoglycoprotein receptor that is expressed exclusively on the surface of hepatocytes. Hepatocyte targeted DPCs display preferential accumulation in liver hepatocytes.

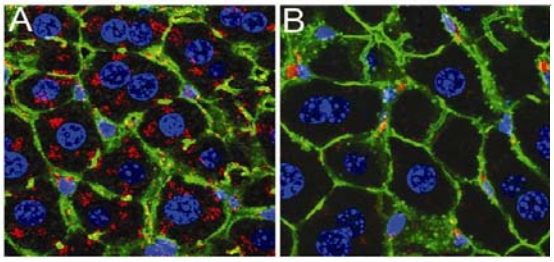

Figure 2: DPCs containing the NAG ligand are targeted to liver hepatocytes in mice. Image A displays NAG-DPCs; Image B displays Non-targeting control Glucose-DPCs. Red = DPCs; Blue = cell nuclei; and Green = cell membranes.

More recently, methods have been developed to attach other classes of ligands, such as glycans, peptides, small molecule drugs, nucleic acids, lectins, and antibodies. The stoichiometry of ligand to polymer to siRNA can be controlled during DPC formulation to obtain optimal targeting properties. These advances open up the possibility of targeting DPCs to a wide range of cell-types outside the liver, including tumors. In fact, extensive *in vivo* biodistribution studies have demonstrated that using antibody-targeted DPCs, >30% of the injected dose per gram of tissue accumulates in the tumor of a colon carcinoma model, an amount almost unheard of for conventional chemotherapeutic drugs.

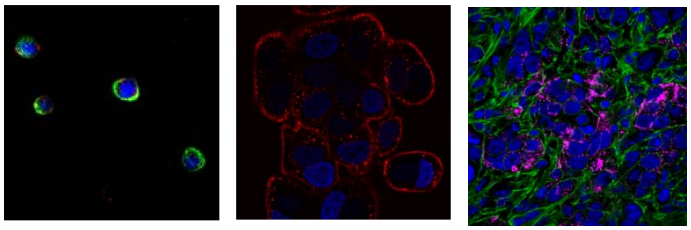

Figure 3: Ligand-mediated DPC delivery to other cell types. (Left) – Targeting to leukemia cells in vitro using small molecule ligand attached to the DPC. Antibody-mediated DPC targeting to tumor cells in vitro (Center) and in vivo (Right). Red = DPCs; Blue = cell nuclei; Green = cell membranes.

DPCs for Delivery to Hepatocytes

Hepatocytes, the key parenchymal cells of the liver, are a particularly attractive target cell type for siRNA delivery given their central role in several infectious and metabolic diseases. Latest generation DPCs are remarkably efficacious in rats and non-human primates with ED80 values of ~0.1 mg/kg siRNA after a single dose. Increasing the dose two-fold in non-human primates results in >99% knockdown with a duration of effect of nearly 7 weeks. This is a 10-fold increase in efficacy compared to first generation DPCs containing PBAVE polymer. Latest generation DPCs are also better tolerated and have therapeutic indices of >10 in non-human primates as calculated from ED80 and NOAEL values. The magnitude of the safety margin in non-human primates is unprecedented in the therapeutic RNAi field and positions DPC technology as a frontrunner for siRNA delivery to liver.

DPCs for Delivery to Tumors

Our DPC cancer delivery program aims to develop the optimal components for targeting DPC to tumors. This includes identifying ligands for efficient targeting, screening polymer libraries for the most potent polymer and enhancing tumor uptake by modulating the pharmacokinetic properties of the DPC.

DPCs for several types of tumors are currently under development. Hepatocellular carcinoma (HCC) has been one of our focus areas for proof of concept studies. Gene knockdown of 40-50% has been achieved with a single dose of tumor directed DPCs in a mouse orthotopic HCC tumor model. These results equal or surpass those achieved with other best in class siRNA delivery platforms and validate our overall strategy for tumor targeted DPC delivery. Our current focus is on further improving delivery to tumors, gene knockdown efficacy and the therapeutic index by optimizing individual DPC components in animal models.

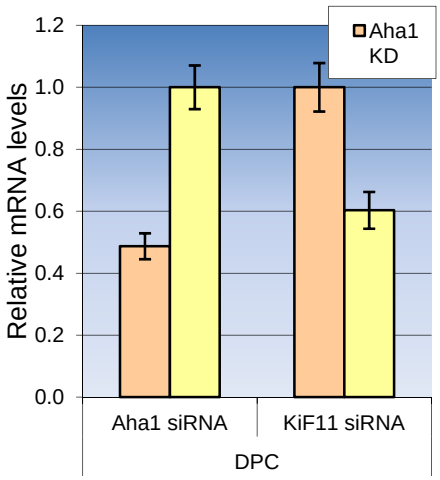

Figure 4: Target gene knockdown in an orthotopic mouse model of human HCC using DPCs

Outlook

DPCs promise to be a great leap forward in the field of RNAi therapeutics. Advances in polymer chemistry, masking technology, ligand attachment chemistry, formulation and siRNA optimization make current generation DPCs more efficacious, safer and more easily manufactured than first generation DPCs. Pre-clinical studies using non-human primates reveal that DPCs are highly efficacious for delivery to liver with safety margins beyond those currently achievable by other systems. Further, the modular nature of DPC technology and the ability to optimize individual components within the same platform give it the potential to effectively target cell types outside the liver.