Design and development of effective siRNA delivery vehicles

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As medicines become increasingly complex, their delivery becomes more challenging. To function, medicines must effectively navigate the body and reach their site of action. When the impediments to their delivery become too great, drug delivery vehicles are used. Small interfering RNA (siRNA) is a complex class of medicine. These duplex nucleic acid structures enter the RNA-interference pathway (RNAi) to alter the expression of a specific protein. The obstacles to siRNA delivery as a therapeutic agent are many. The work presented in PNAS by Dong et al. (1) represents an innovative approach toward the design and development of effective siRNA delivery vehicles.

The goal of siRNA-based therapy is to knock down the expression of a specific protein to bring about a specific therapeutic effect. A number of siRNA therapeutics are in clinical trials (2). To exert their function, siRNA sequences must enter the RNAi pathway, which takes place in the cytosol. Two significant obstacles to the use of therapeutic siRNA are their macromolecular and polyanionic composition, which restrict their passive diffusion across the cell membrane into the cytosol. Therefore, to function siRNA therapeutics must be actively transported into the cell, but the fate of actively internalized macromolecules is usually catabolism. The job of an effective siRNA delivery vehicle is to direct and deposit functionally active siRNA to the cytosol of a target cell population. The development of effective siRNA vehicles has been an intensely active area of research for the past decade.

Viruses are naturally evolved and highly effective nucleic acid delivery vehicles with potential clinical utility. However, their intrinsic immunogenicity limits repetitive administration, which motivates the discovery of synthetic nucleic acid delivery vehicles. One approach toward their development is to use the combinatorial synthesis principles and methods that were developed and used previously in the genomic (3), drug discovery (4), materials (5), and pharmaceutical formulation fields (6, 7). Combinatorial methods

permit the design and synthesis of a wide range of structural compositions to help build a greater understanding of how molecular composition correlates to function. By screening large libraries of structures, investigators can expand their knowledge base and identify structure–function relationships to begin working toward structures with the attributes needed to perform a specific function.

There are different ways to screen molecular parameter spaces to probe for structure–function relationships (Fig. 1). “Serendipitous screening,” for lack of a more sophisticated term, implies exploration of structural libraries without a theoretical framework. Although the moniker conjures images of an unflattering Edisonian approach, serendipitous screening has merit when the experimental framework precedes an adequate theoretical basis. The early use of combinatorial approaches toward the identification of more effective nucleic acid delivery reagents began in this way (8).

When structure–function information or a theoretical framework exists, “rational screening” can take place. Rational screening implies a starting point from which the interrogation for structure and function can begin. The approach is used to either improve on—or better understand—the function of existing structures. Additionally, rational screening can be used to recapitulate the activity of a structure through mimicry of function, but with different structural compositions. The work of Dong et al. in PNAS (1) is a prime example of rational screening, using the structure and natural function of lipoproteins as the starting point.

Lipoproteins help to transport lipids and cholesterol into cells and throughout the body, and the liver is a principal player in the natural homeostasis of lipids and cholesterol. Using the structure of lipoproteins as a starting point, Dong et al. (1) investigated the combinatorial synthesis of lipidic structures with cationic headgroups to mimic—or hijack the function of—lipoproteins to transport siRNA to hepatocytes. From a primary

Fig. 1. Fooling Mother Nature. Natural evolution selects for structural functionality in a linear fashion over time. Serendipitous screening searches for structural functionality by parallel screening of structural libraries, thereby speeding up time. Rational screening iteratively searches for structural functionality starting from a theoretical framework and enhances fundamental understanding by experimental validation and refinement of theory.

screen, the investigators identified a lead structure that was used to guide the synthesis of a second structural library, from which a lead composition was formulated. The result of their rational screen led to unprecedented siRNA delivery efficacies and cellular specificities. Perhaps most impressive is that the effects are consistent across species, from rodents to nonhuman primates.

The liver is a foreign-body scavenger for the blood stream, which promotes the belief that it is an “easy” organ to target. However, to target hepatocytes with high fidelity requires finesse because the resident liver macrophages so easily scavenge nonself structures. Early work by Kopecek and colleagues investigated the asialoglycoprotein receptor to target polymer–drug conjugates (9, 10) to hepatocytes with high efficacy. The work of Dong et al. (1) leverages the natural lipoprotein receptors resident on hepatocytes to achieve cell-specific protein knockdown efficacy that is orders-of-magnitude more potent than siRNA targeted to the liver using cholesterol (11), cholesterol lipoprotein-associated complexes (12), or α -tocopherol (13). In

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addition to high potency, hepatocyte selectivity was also orders-of-magnitude greater relative to other liver-resident cells, notably macrophages and endothelial cells. The results are impressive.

The paper by Dong et al. (1) is one example of how the interplay between experimental sophistication and theoretical development can lead to fundamental discoveries and advance medicine. The iterative

synthesize-screen-resynthesize-repeat approach defines “synthetic evolution,” and its use within a theoretical framework is a powerful way to help drive the discovery and development of new materials.

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