DIRECTED EVOLUTION OF NEW ADENO-ASSOCIATED VIRAL VECTORS FOR CLINICAL GENE THERAPY

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Key Words: gene therapy, viral vectors, AAV, directed evolution, manufacturing

Gene therapy – the delivery of genetic material to the cells of a patient for therapeutic benefit – has been increasingly successful in human clinical trials over the past decade, and there are numerous FDA-approved gene therapies. The most successful gene delivery vehicles, or vectors, are based on adeno-associated viruses (AAV); however, vectors based on natural versions of AAV face a number of delivery barriers that limit their efficacy and will thus preclude the extension of these successes to the majority of human diseases. Furthermore, efforts to overcome these barriers simply by increasing dose incur major manufacturing challenges and risk inflammatory responses within patients. Such delivery limitations arise since the parent viruses upon which these vectors are based were not evolved by nature for our convenience to use as human medicines. Unfortunately, due to the highly complex mechanisms of virus-host interactions, there is currently insufficient mechanistic knowledge to enable rational design to be sufficiently successful in creating new vectors. As an alternative, however, we developed the concept of using directed evolution to engineer highly optimized variants of AAV for a broad range of cell and tissue targets. Directed evolution involves the iterative genetic diversification of a biomolecule to create a gene pool and functional selection to isolate variants with optimal properties. Using this approach, we have engineered AAV variants with greatly improved delivery efficiency to multiple organs including the retina; lung, and muscle; targeted delivery to specific cell types; and the capacity to evade immune responses. Our novel AAV variants are currently used in 8 human clinical trials involving delivery to the retina, heart, and lung. Furthermore, we have been employing genomewide CRISPR/Cas9-based screening to identify genes whose overexpression in manufacturing cells results in increased AAV production. The integration of high throughput screening technologies to improve AAV efficacy and production can enable a broad range of basic and therapeutic gene delivery applications.