Drug discovery and target identification platform technology using random shRNA-expressing library

shRNA library of 3 million sequences for identification of small-RNA therapeutic candidates, new targets and pathways, as well as conventional chemical-compound drugs in cell-culture disease models.

Inventor
Robert B. Wilson, MD, PhD

INTELLECTUAL PROPERTY
- WO2007103365 (nationalized in US, EU, CA, JP, AU, HK)
- WO2015020993

REFERENCE MEDIA
- Wang et al., PLoS One. 2008; 3(9):e3171
- Cotticelli et al., J Biomol Screen, 2015; 20(9):1084-90

DESIRABLE PARTNERSHIP
- License
- Co-development

shRNA Drug Discovery
Problem
RNA interference (RNAi) using short hairpin RNA (shRNA) is commonly used to inhibit gene expression. shRNA-expressing libraries may have important applications in identifying RNA molecules/sequences with specific biological activity and thus therapeutic implications. Typically, shRNA libraries are limited to sequences that target single mRNAs. Because 7-nucleotide “seed” sequences within shRNAs are sufficient for partial inhibition of target mRNAs, shRNAs are inherently promiscuous. Thus, the single-gene-targeting approach is complicated by off-target effects, which diminish therapeutic indices, and fails to take advantage of multi-gene targeting, which enhances potency.

Solution
To challenge the single-gene-targeting paradigm of small-RNA-based therapeutic initiatives, Dr. Wilson’s lab designed, synthesized, validated, and now further optimized shRNA-expressing libraries that are completely random at the nucleotide level. Three million shRNAs can be screened in a single tissue-culture flask, selecting for the desired phenotype with “hit” sequences retrieved by PCR. Because the libraries are completely random, the screens are unbiased: favorable cell phenotypes reveal which shRNAs are most effective and least toxic. This approach allows identification of sequences that target multiple genes and/or act through non-canonical mechanisms.

Discovery of Drugs, Targets, and Pathways
Problem
Traditional drug discovery process involving high-throughput screening is labor-intensive, expensive, often ineffective, and infeasible when cell-culture disease models are unsuitable for microtiter-plate formats.

Solution
Random shRNA library screening can be combined with bioinformatic pattern analyses of hit sequences to identify targets, pathways, and conventional chemical-compound therapeutic candidates, bypassing in vivo delivery issues. Thus, each phenotypic screen has the potential to identify, (i) small-RNA therapeutic candidates, (ii) conventional, chemical-compound therapeutic candidates, (iii) target candidates for conventional drug development, and (iv) information on pathways relevant to disease mechanisms.
Advantages

- Screening, by phenotypic selection, of 3 million shRNAs in a single tissue-culture flask
- Screening for efficacy, and lack of toxicity, simultaneously
- Hit-optimization by random mutagenesis and re-screening
- Translation, using bioinformatics, of gene-expression profiles of “hit” shRNAs to existing drugs
- Inexpensive, simplified, fast method compared to high throughput screening
- Ability to run positive and negative (cancer) screens
- Utilization of cell-culture disease models unsuitable for microtiter-plate formats

Applications

- Identification of shRNA sequences that confer phenotypes of interest or modulate specific biological parameters
- Identification of novel targets, pathways and existing drugs through bioinformatic pattern analyses
- Development of novel therapeutics and biologic tools for a variety of diseases, including, e.g.
  - Viral illnesses, by selecting for cell survival in viral cell-culture models
  - Cystic Fibrosis, by selecting for increased surface expression of F508del CFTR
  - TRAIL-insensitive Malignancies, by selecting for increased surface expression of the TRAIL receptor
  - Hypercholesteremia, by selecting for increased surface expression of the LDL receptor
  - Parkinson Disease, by selecting for increased expression of PGC1-alpha
  - Type II Diabetes, by selecting for protection against inducers of ER stress and the unfolded protein response (UPR)

State of Development

- Synthesized, validated, and optimized library of 3 million sequences
- Identified shRNAs protecting an IL3-dependent cell line from IL3 withdrawal
- Identified shRNAs that reverse phenotypic defects of Friedreich ataxia (FA) fibroblasts
- Identified novel targets and existing drugs that reverse phenotypic defects of FA fibroblasts