Therapeutic Discovery for Friedreich Ataxia Using Random shRNA Selection

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Therapeutic discovery for Friedreich ataxia using random shRNA selection

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Abstract

We screened a 300,000-clone, random shRNA-expressing library and identified shRNA sequences that reverse the decreased growth/survival phenotype of primary Friedreich ataxia (FA) fibroblasts grown in mitochondrial stress media. One of the hit sequences, gFA2, increases frataxin expression 12-fold, either as a vector-expressed shRNA or as a transfected shRNA. We randomly mutagenized gFA2 to create a gFA2 variant sub-library. We screened this sub-library in primary FA fibroblasts and identified two gFA2 variants, gFA2.8 and gFA2.10, that further increase frataxin expression. Microarray analyses of primary FA fibroblasts expressing another hit shRNA, gFA11, revealed alterations in >350 mRNAs. Bioinformatic pathway analysis indicated significant changes in mRNAs involved in cyclin-dependent kinase (CDK) and CDK-related cell cycle pathways. We confirmed significant changes in ccNKd cell cycle genes induced by gFA11 biochemically. Ingenuity Pathway Analysis revealed that inhibition of a known transcription factor, or treatment of cells with a previously studied chemical compound, induced a distinctly different, similar pattern of gene expression to that induced by gFA11. Inhibition of the transcription factor using a directed siRNA in primary FA fibroblasts, as well as treatment of the cells with the chemical compound, recapitulated the phenomena observed by gFA11, namely reversal of decreased growth/survival in mitochondrial stress media. We are currently planning similar microarray and bioinformatic analyses of the optimized versions of gFA2. Combined with microarray analyses and bioinformatic pattern-matching, our random, shRNA library screens potentially yield: 1) small RNA therapeutic candidates, 2) conventional chemical compound therapeutic candidates, and 3) elucidation of disease mechanisms, which may inform additional therapeutic initiatives.

Materials and Methods

Identification of Drug Target and Mechanisms

Candidate 1: gFA11

FRDA ataxia (FRDA) is an autosomal recessive neuro and cardio-degenerative disorder, with a prevalence of approximately 1 in 40,000 in European populations. Recent reviews include those by Koeppen and Mazurkiewicz,1 Collins,2 and Gomes and Santos.3 FRDA is characterized by progressive ataxia of all four limbs, dysarthria, areflexia, sensory loss, and muscle fatigue. FRDA is caused by mutations in the nuclear gene, FXN, which encodes the highly conserved protein frataxin. Most disease alleles harbor a GAA repeat expansion in the first intron, which results in decreased transcription. Frataxin localizes primarily to the mitochondrial matrix, where it chaperones iron and regulates the iron-sulfur cluster (ISC) assembly complex.

Candidate 2: gFA2

Materials and Methods

GSEA and qMENA8 FA cells were from Cornell. Patient-derived cells 401-7 were a gift of Dr. David Lynch (Children’s Hospital of Philadelphia, PA, USA). Microarray analysis used the Affymetrix GeneChip Human Gene 2.0 ST array. Luminex analysis was performed by the Human Immunology Core Facility at University of Pennsylvania using the Milliplex Panel HCYTOMAG-60K kit (Millipore). For the Bioinformatic analyses, PA was run through the Penn Genomic Analysis Core; we used the free online available version of the Database for Annotation, Visualization and Integrated Discovery (DAVID) v.6.7 and Gen Set Enrichment Analysis from the Broad Institute (MA).

Identification of Drug Target and Mechanism

G.S.E. analysis of microarray data

Enrichment in phenotype: gFA11 (3 samples) 403 / 577 gene sets are upregulated
115 gene sets are significant at FDR < 25%
60 gene sets are significantly enriched at nominal p-value < 1%

The pathways identified by GSE Analysis are currently under investigation.

References