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MOVEMENT DISORDERS

Targeted RNA or BDNF gene transfer protects against frataxin deficiency

> BDNF overexpression countered the deficits in motor co-ordination

Friedreich ataxia is a genetic disorder caused by a GAA expansion in intron 1 of the *FXN* gene, which encodes frataxin. In two recently published studies, the pathological consequences of this *FXN* mutation have been successfully counteracted in *in vitro* and *in vivo* models with the use of different approaches, each with therapeutical potential.

In the first study, David Corey and colleagues built on existing evidence that the GAA expansion in *FXN* leads to formation of an R-loop, in which pre-mRNA binds to template DNA, that interferes with transcription. The researchers aimed to block this transcriptional suppression by targeting the repeat expansion.

"We had previously shown that we could target expanded CAG repeats to decrease expression of Huntingtin, ataxin-3 and atrophin-1," explains Corey. "We decided to target *FXN* expression because of the unmet need for agents that selectively activate *FXN* expression."

The team first introduced synthetic anti-GAA duplex RNA molecules into patient-derived cells that had the *FXN* mutation. Introduction of the anti-GAA RNA increased expression of frataxin by up to sixfold. Chromatin immunoprecipitation showed that the RNA duplex reversed histone acetylation and methylation that is associated with the GAA expansion, thereby activating gene expression. Single-stranded anti-GAA locked nucleic acid molecules had the same effect, whereas control non-complementary strands did not, confirming that binding of the synthetic RNA to the pre-mRNA is responsible for gene activation.

"Other labs have reported that histone-modifying agents could activate *FXN* expression, but not in a gene-selective way," says Corey. "We used synthetic duplex RNA and single-stranded oligonucleotides because they permit sequence-specific recognition of target RNA."

The authors believe that their findings are a starting point for the development of RNA-based drugs, and are looking to take the next step. "We look forward to helping laboratories in acadaemia or industry move forward into animal model testing," says Corey.

The second study, led by Javier Diaz-Nido, did not involve targeting the *FXN* gene, but aimed to block the apoptosis that, as previously shown by the same research group, is a result of frataxin underexpression that leads to neurodegeneration. "As it is well known that neurotrophic factors are potent suppressors of neuronal apoptosis, we decided to check whether they were able to protect neurons from death triggered by frataxin gene silencing," explains Diaz-Nido.

The researchers initially created an *in vitro* model of frataxin underexpression, in which small hairpin RNA against frataxin was delivered by lentiviral infection of cultured neurons. They showed that the presence of recombinant neurotrophins, particularly brain-derived neurotrophic factor (BDNF), reduced activity of caspase-3 (a marker of apoptosis) that resulted from frataxin underexpression, and increased cell viability. The same benefit resulted from the more clinically relevant approach of lentiviral gene transfer to produce overexpression of BDNF.

Once the principle had been proved in cultured cells, Diaz-Nido and his team tested the same approach in mice. They used lentiviral infection to reduce frataxin expression, and showed that co-delivery with the gene that encodes BDNF protected cerebellar cells against apoptosis. Furthermore, BDNF overexpression countered the deficits in motor co-ordination that were seen in mice with frataxin underexpression but no BDNF overexpression.

Diaz-Nido points out that their results are some way from clinical application, but says that they suggest new therapeutic approaches to Friedreich ataxia. "For instance, one approach being considered for Friedreich ataxia is gene therapy, but efficient delivery of the viral vectors is still an issue. We plan to test whether there is a synergy between two gene therapy strategies: one based on frataxin gene replacement and the other based onneurotraphic factor gene delivery," Diaz-Nido concludes.

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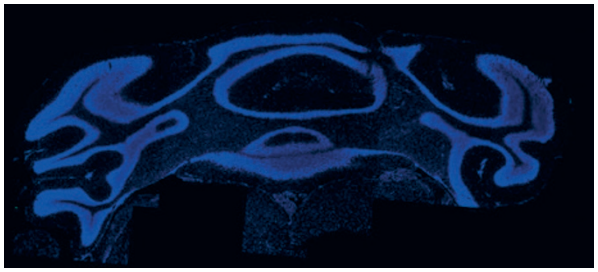

Coronal section of a mouse cerebellum, into which small hairpin RNA was delivered to suppress frataxin expression. Image courtesy of Javier Diaz-Nido.

ORIGINAL ARTICLES Li, L. *et al.* Activating frataxin expression by repeat-targeted nucleic acids. *Nat. Comm.* http://dx.doi.org/10.1038/ncomms10606 | Katsu-Jiménez, Y. *et al.* Gene transfer of brain derived neurotrophic factor (BDNF) prevents neurodegeneration triggered by frataxin deficiency. *Mol. Ther.* http://dx.doi.org/10.1038/mt.2016.32