Targeting toxic RNA

By Tracey Baas, Editor

Researchers at the **University of Rochester** have used an antisense molecule to reverse muscular defects in mice with myotonic dystrophy type 1.¹ The approach represents a new way to attack the most common form of adult muscular dystrophy and may be applicable to other diseases that are also characterized by expanded RNA repeats.

Myotonic dystrophy type 1 (DM1) is an RNA-mediated, dominantly inherited disease caused by long triplet repeat regions of CUG in the noncoding region of the RNA that is translated into dystrophin myotonic protein kinase (DMPK). Whereas healthy people have about 5–30 copies of the triplet repeat, DM1 patients have hundreds to thousands of copies.

The repeat regions produce problems because they have a high affinity for muscleblind-like 1 (MBNL1), a splicing regulator involved in the expression of several muscle proteins including muscle-specific chloride channel (CIC-1). The regions essentially sequester MBNL1, which results in low intracellular levels of MBNL1 and incorrect splicing of CIC-1. This, in turn, leads to the formation of aberrant chloride channels and muscular dysfunction.

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—Charles Thornton,
University of Rochester

Previous strategies for treating DM1 have sought to cause overexpression of MBNL1,² but the Rochester group took a different tack: stop MBNL1 from binding to the RNA repeats. The hypothesis was that molecules that bind to expanded RNA in an untranslated region would inhibit the RNA-protein interaction and alleviate disease pathology (see **Figure 1, “Effect of RNA expansion on disease pathology and therapeutic intervention”**).

A team led by Charles Thornton, associate professor of neurology at the university’s medical center, injected DM1 mice with a 25-nucleotide-long antisense morpholino, dubbed CAG25, which targeted the CUG repeats. The treatment resulted in Mbnl1 redistribution in muscle fibers and correction of aberrant splicing of CIC-1 and three other muscle-related proteins.

CAG25 also restored chloride channel activity and reduced myotonia in the mice. Data were published in *Science*.

Morpholinos are DNA analogs that have standard nucleic acid bases but are bound to morpholine rings instead of deoxyribose rings and are linked through phosphorodiamide groups instead of phosphates. The result is that morpholinos are more resistant to degradation than DNA.

“The new study shows tremendous promise for treating triplet-expansion diseases and may have applications beyond the precise CUG-repeat RNA target to which it was applied,” said

Benjamin Miller, an associate professor of biochemistry and biophysics at Rochester. Miller has collaborated with Thornton to identify small molecules that inhibit the CUG-repeat RNA of DM1 from interacting

Figure 1. Effect of RNA expansion on disease pathology and therapeutic intervention. Molecules targeting RNA repeats could be useful for treating diseases in which expansion of these repeats within noncoding or coding regions of specific genes results in a high level of detrimental RNA-protein interactions.

[a] Researchers at the University of Rochester showed that using an antisense molecule targeting RNA repeats in untranslated regions (UTRs) of the *dystrophin myotonic protein kinase* (DMPK) gene led to reduced myotonia in mice with myotonic dystrophy type 1 (DM1). By blocking interactions between these repeats and the splicing regulator muscleblind-like 1 (MBNL1), the researchers found that the MBNL1 protein was redistributed in muscle fibers and restored the activity of muscle-specific chloride channel (CIC-1). This approach also could be useful for treating diseases with a similar pathology, such as myotonic dystrophy type 2 (DM2).

[b] Within a translated gene region, expanded RNA can lead to mutant protein production, which contributes to disease pathology. However, researchers suspect that these RNA repeats within the coding regions of a specific gene may also lead to unwanted RNA-protein interactions that contribute to disease progression. Thus, targeting these repeats could inhibit unwanted RNA-protein interactions as well as repress the translation of the mutant protein. Diseases in which RNA repeats are found within a specific gene include spinocerebellar ataxia type 3 (SCA3) and Huntington’s disease (HD).

with MBNL1. That work was published last year in the *Journal of the American Chemical Society*.³

“In theory, inhibiting the interaction of proteins with toxic RNA could be useful for any RNA-dominant disease caused by expanded repeats,” Thornton told *SciBX*. “Other diseases caused by expanded repeats within untranslated regions are rare, but myotonic dystrophy type 2 (DM2) might be a logical disease to target,” he added. “There is the possibility that the method could be useful for targeting expanded repeats within coding regions of RNA, such as in Huntington’s disease and spinocerebellar ataxia type 3.”

But it is unknown whether interactions between regulatory proteins and expanded RNA in translated regions contribute to Huntington’s disease (HD) and spinocerebellar ataxia type 3 (SCA3) (see **Figure 1**, “Effect of RNA expansion on disease pathology and therapeutic intervention”).

Thornton has taken initial steps to find out—he has worked with Matthew Disney, assistant professor of chemistry at the **State University of New York at Buffalo**, on identifying small molecules that bind the repeat triplets of *ataxin 3* (*ATXN3*), the gene underlying SCA3, *in vitro*.⁴ The molecules have not yet been tested for *in vivo* efficacy.

According to Ryszard Kole, SVP of discovery research at **AVI BioPharma Inc.**, the use of antisense morpholino oligomers to target disease-causing RNA repeats may be particularly well suited for muscle-related conditions.

“Current research points toward the ability to deliver RNA repeat-binding morpholino oligomers to muscles, but there are scarce data suggesting that these molecules could be delivered to brain for neurological diseases” such as HD or SCA3, he said. “Small molecules may be different in this respect.”

In addition, Kole thinks that therapeutics targeting RNA repeats will

need to be effective in systemic—rather than intramuscular—delivery. The reason is that DM1 weakens skeletal muscles in all parts of the body, including internal ones like the diaphragm, which cannot be injected directly.

Also, the morpholino described in the *Science* paper required localized intramuscular injection and also *in vivo* electroporation, which is an inefficient procedure that requires the application of high voltages.

AVI’s AVI-4658 morpholino oligomer is in Phase Ib testing for Duchenne muscular dystrophy (DMD). The company’s next-generation peptide-conjugated morpholino oligomers (PPMOs) undergo more effective systemic delivery, thus avoiding intramuscular injection and electroporation. Recent data have shown that PPMOs improve quadriceps muscle, diaphragm muscle and heart muscle in a mouse model of DMD.⁵

A patent application has been filed for the findings in the *Science* paper and is available for licensing through the University of Rochester.

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