Team introduces new method to closely model diseases caused by splicing defects

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A team led by scientists at Cold Spring Harbor Laboratory (CSHL) has developed a new way of making animal models for a broad class of human genetic diseases – those with pathology caused by errors in the splicing of RNA messages copied from genes. To date, about 6,000 such RNA "editing" errors have been found in various human illnesses, ranging from neurodegenerative disorders to cancer.

The new modeling approach can provide unique insights into how certain diseases progress and is likely to boost efforts to develop novel treatments. It was tested successfully by the CSHL team, led by Professor Adrian Krainer, Ph.D., in collaboration with scientists from Isis Pharmaceuticals, in mouse analogs of human spinal muscular atrophy (SMA), a motor-neuron disease that is the leading genetic cause of childhood mortality. The results are detailed in a study published today in *Genes & Development*.

The modeling method is called TSUNAMI (shorthand for targeting-splicing using negative ASOs to model illness). The study demonstrates it can be used in illnesses with pathology associated with the missplicing of pre-mRNAs – unedited RNA molecules that bear the messages encoded in genes which provide instructions for cells to manufacture specific proteins.

Correcting splicing errors in SMA

A cellular machine called the spliceosome routinely snips non-essential bits called "introns" out of every pre-mRNA molecule that carries a copy of a gene's instructions. All that should remain after the spliceosome has done its work is a string of spliced-together "exons," the protein-encoding portions of the message. These edited mRNA messages are subsequently read by ribosomes, the cellular factories where proteins are synthesized.

In SMA and some other human illnesses, pathology can be traced to errors in the pre-mRNA editing process. In SMA, it is caused either by a severe mutation in a gene called SMN1 ("survival of motor neuron-1") or by that gene's complete absence in an affected individual's genetic material. The SMN protein normally encoded by the gene is essential for motor neuron development. Humans have a second, similar gene called SMN2, but it is a poor backup. Because of an error in the splicing of its pre-mRNA, the SMN2 gene, when expressed, typically produces only a fraction of the SMN protein needed by motor neurons. This is critical in the first stages of life when the body and muscles are still developing.

While the level of the "backup" gene's protein output varies in individuals with spinal muscular atrophy, resulting in pathology of varying intensity, Krainer -- a leading expert on splicing -- and his collaborators have succeeded in recent years in devising a method of getting SMN2 to produce therapeutic amounts of protein, enough to reverse pathology in both mild and severe mouse analogs of the disorder.

To achieve this they synthesized tiny snippets of RNA called ASOs (antisense oligonucleotides) and injected them into the cerebrospinal fluid of mice carrying a human SMN2 transgene (i.e., a gene not native to mice). This enabled them to get the therapeutic ASOs through the so-called blood-brain barrier, to reach cells throughout the central nervous system. ASOs are configured to attach at highly specific spots in pre-mRNAs, where, by design, they can inhibit activators or repressors of

the splicing process. Krainer's team synthesized an ASO that corrected the SMN2 splicing error and gave rise to therapeutic amounts of SMN protein. Importantly, the ASO was shown to be stable in the body as well as persistent, the effects of a single injection lasting at least half a year in mice.

TSUNAMI's 'negative ASOs': therapy in reverse

A version of this therapeutic ASO is now being tested in Phase 1 human trials. But even as the tests proceed, Krainer and colleagues have worked on getting the splice-correction method to work in reverse: using a "negative ASO" to cause or exacerbate disease pathology in neonatal mice, with the approach they call TSUNAMI.

The team synthesized ASOs that by virtue of their chemical sequence target a different site on the pre-mRNA of the human SMN2 gene, one that exacerbates missplicing. When these negative ASOs were injected into the ventricular brain region of mice engineered to have four copies of the SMN2 transgene, and lacking the mouse's own Smn gene, SMA symptoms became more severe than in control animals. With four copies of the transgene, untreated mice only have very subtle symptoms. But when the negative ASOs were injected immediately following birth, severe SMA-like pathology was observed to develop in a dose-dependent and progressive fashion [see movies]. The team also demonstrated that it could "rescue" these mice by injecting them with the therapeutic ASO they had previously shown can correct the SNM2 splicing error.

"This amounts to a proof of principle," Krainer says. "By using TSUNAMI we are able to dissect pathogenesis mechanisms, including spatial and temporal features of disease onset and progression. We are also able to observe the impact of candidate therapeutics in the same manner, both as a function of where they have their impacts in the body and when."

In the experiments they report today, the team describes an aspect of SMA pathology not previously known. It is pathology, Krainer hypothesizes, that is secondary to the disorder's primary pathology. The sick mice find it more and more difficult to move about as their illness progresses; they become unable and perhaps also uninterested in feeding. The starvation that ensues is a consequence of the primary pathology; but it also induces new elements of pathology. In this case, the team observed that the "starved" mice now began to suffer from other problems, including additional SMN2 splicing errors, which presumably contribute to an acceleration of pathology as the disease enters its terminal stages.

TSUNAMI will provide new modeling alternatives

Kentaro Sahashi, a postdoctoral fellow who is first author on the new paper and a member of the Krainer lab, stresses that TSUNAMI is offered as an alternative and complement to other methods of making animal models of human illness. By knocking out genes, or modulating their expression patterns in a variety of ways, molecular biologists have made great strides in understanding human cancers, for instance. "These are very powerful technologies," Sahashi observes, "and TSUNAMI does not in any way replace them. Rather, we think of it as providing specific advantages in modeling certain illnesses."

In addition to its ability to reproduce the spatial and temporal patterns of pathology, TSUNAMI has the advantage, in splicing-related illnesses, of inducing pathology by mimicking precisely the mechanism that gives rise to the illness in people.

It can also help identify clinical biomarkers for illnesses. "You can generate a disease and rescue it; you can generate a series of mice treated in different ways. And using mass spectrometry and other techniques, you can look for biomarkers," explains Krainer. "Then, of course, you

have to test to see if these putative markers are clinically relevant. This is a powerful technology for doing this sort of work, and not only in SMA."

Krainer further notes that negative ASOs can be used to target pre-mRNAs of genes that are naturally occurring in an animal – rather than of a human transgene, the target in the experiments reported today. This way, he says, "one should be able in principle to generate splicing-associated disease models in virtually any wild-type species."

Krainer's lab at CSHL may soon turn to another illness, familial dysautonomia, caused by a splicing error. But splicing errors also crop up in many other illnesses, from cystic fibrosis to cancer. "We can't possibly fix every splicing defect found in these diseases, but we can certainly cause something analogous to them and learn from the models we make," Krainer says.

More information: "TSUNAMI: an Antisense Method to Phenocopy Splicing-Associated Diseases in Animals" appears online ahead of print in *Genes & Development* August 14, 2012.

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