Researchers identify drug candidate for treating spinal muscular atrophy

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A chemical cousin of the common antibiotic tetracycline might be useful in treating spinal muscular atrophy (SMA), a currently incurable disease that is the leading genetic cause of death in infants. This is the finding of a research collaboration involving Adrian Krainer, Ph.D., of Cold Spring Harbor Laboratory (CSHL) and scientists from Paratek Pharmaceuticals and Rosalind Franklin University of Medicine and Science.

SMA is caused by mutations in a gene called Survival of Motor Neuron 1 (SMN1), resulting in a decrease in the levels of SMN protein in the motor neurons of the spinal cord - the cells that control muscle activity. Without the protein, these neurons degenerate, and infants born with the mutations progressively lose the ability to move, swallow, and breathe. There are no approved therapies for the treatment of SMA, which affects approximately 1 in 6,000 babies born in the United States.

The new molecule boosts the levels of SMN protein in cells by fixing a mistake in a cellular processing mechanism called RNA splicing. In a study that appears in the journal *Science Translational Medicine* on November 4th, the scientists report this fix in both mouse models of SMA, as well as in cells isolated from SMA patients.

Unlike previously identified molecules that stimulate SMN production, the tetracycline-like compound is a unique therapeutic candidate in that it is a small molecule that specifically alters RNA splicing by directly targeting the splicing reaction.

Further collaborative research will focus on pre-clinical drug development, and is being supported by a five-year, multi-million dollar cooperative agreement from the National Institute of Neurological Disorders and Stroke (NINDS) and by the Families of SMA funding program.

Correcting RNA splicing

The drug candidate targets the splicing of a gene called SMN2, which is essentially a back-up copy to the SMN1 gene that's mutated beyond repair in SMA patients. SMN2 doesn't compensate for the loss of SMN1, however, because it produces too little functional protein. Most of the protein that is produced is missing a single important piece, without which the protein rapidly degrades.

The omission of this piece of the SMN protein is due to a defect in the way in which the cell's splicing machinery processes the RNA copied from the DNA of the SMN2 gene. During splicing, a complex of enzymes snips out of the RNA certain unneeded pieces called introns. Normally, the remaining, necessary pieces called exons are spliced back together, and this edited RNA molecule is then converted into functional protein.

In the case of the SMN2 gene, however, the splicing machinery skips an exon. So Krainer and his collaborators searched for ways to alter splicing so that this missing piece, the 7th exon, is included in the final RNA copy.

The researchers focused their search on a class of molecules that are chemical variants of tetracycline because this class of chemicals is known to latch on to RNA and modify splicing, and is less toxic than others that can do the same.

In an experimental system that tests the effect of molecules solely on splicing, the scientists screened a number of tetracycline derivatives from Paratek's chemical library. The screen revealed that a molecule named PTK-SMA1 is highly efficient at altering splicing such that exon 7 is included.

PTK-SMA1 as a potential therapeutic

The researchers confirmed that this effect of PTK-SMA1 on RNA splicing and exon inclusion ultimately results in increased levels of full-length and functional SMN protein. The compound boosted protein levels in cells isolated from SMA patients and cultured in lab dishes. The scientists also proved its ability to work in vivo by injecting it into mice carrying a human SMN2 gene. The mice showed more than a 5-fold increase in human SMN protein levels within a week of treatment.

"PTK-SMA1 is the only small molecule known to specifically alter RNA splicing by directly and solely targeting the splicing reaction," says Krainer. Other molecules that affect splicing also affect other cellular processes, thus diluting their potency, and potentially increasing the risk of side effects. PTK-SMA1 has the added advantage of being a derivative of tetracyclines, which are nontoxic and have demonstrated safety in humans.

The team is excited about having such a promising therapeutic candidate for SMA treatment and plans to next focus on two key issues: finding out exactly how PTK-SMA1 redirects RNA splicing and finding a way to get it across the blood-brain barrier and into the affected neurons in the spinal cord.

More information: "Tetracyclines That Promote SMN2 Exon 7 Splicing as Therapeutics for Spinal Muscular Atrophy" appears online ahead of print in *Science Translational Medicine* on November 4th.

Source: Cold Spring Harbor Laboratory (news : web)