Advances in RNA interference – Delivery and Allele-Specific Silencing

Many researchers now believe that the cure for Huntington's Disease will involve silencing the mutant gene. The HD community was electrified by Dr. Beverly Davidson's groundbreaking research with a mouse model of spinocerebellar ataxia, another autosomal dominant genetic neurodegenerative disorder in 2004 and again in 2005 when she successfully silenced the HD gene in the N171-82Q mouse model of Huntington's Disease using RNA interference.

RNA interference can be traced by to a serendipitous finding in plant science in 1990. A botanist was attempting to make a deeper purple petunia and inserted an extra copy of the gene encoding for purple coloration. Instead of achieving his goal, he found that the petunias were white. The reason remained a mystery until 1998 when researchers discovered that the addition of the gene activated a cellular defense mechanism. The cell reacts as if the new RNA is from an invading virus and launches an attack that silences both the new RNA and the original RNA, in this case silencing all of the color genes. It was quickly discovered that RNA interference also works in animal cells and by 2002 scientists began researching the potential for treating human diseases caused by a dominant gene.

There have been encouraging advances in safety and effectiveness in recent years. The biggest challenges are delivery and the need for allele specific silencing.

Since short interfering RNA doesn't cross the blood brain barrier, the method of delivery in animal models has been intrusive, requiring injections directly into the brain using stereotaxic surgery. This method would be too intrusive for treating human beings and the delivery would be too localized to be effective.

In 2007, Dr. Davidson and colleagues reported on a new method of delivery for siRNA. They attached siRNA to a peptide derived from the rabies virus glycoprotein.since it is known to cross the barrier into the brain. They were able to achieve a 50 percent knockdown of the target gene. Repeated administration did not induce inflammation nor did it create antiviral antibodies, suggesting that this procedure will be a safe one. The authors discuss ways to enhance efficacy in further research.

In addition to delivery, another issue has been the question of whether it would be harmful to silence the normal huntingtin's protein gene along with the HD gene. When the RNAi research started, it was already known that the huntingtin's protein is essential for normal fetal development. As the research has advanced, it has become clear that the huntingtin's gene has important functions in the adult brain as well. These include the production of brain-derived neurotropic factor and inhibiting caspase 3. Most people with Huntington's Disease have a normal gene as well as an HD gene. Both genes are expressed which means that both types of protein are produced. Silencing both genes would mean the loss of the normal huntingtin's protein as well as the HD protein and would likely result in some pathology.

Allele-specific silencing would seem to be the answer, but short interfering RNA can't distinguish between alleles based on the number of CAG repeats. In the mouse study, the mouse's own normal huntingtin's protein gene was sufficiently different from the engineered HD gene that it was not silenced by RNA interference.

In a newly published article, Coalition for the Cure researcher Robert M. Friedlander and colleagues used two cell models to show that it is possible to develop a short interfering RNA that distinguishes between the normal and the HD gene based on a polymorphism, a mutation that occurs elsewhere on the gene. The polymorphism, the deletion of one of four tandem GAG repeats, is more commonly found on the HD gene than on the normal gene.

Only some HD patients carry this particular polymorphism. However, if a patient carries a different polymorphism, it should be possible to develop an appropriate siRNA that would distinguish between the normal and HD alleles.

What about individuals with two Huntington's Disease genes? This situation is rare in the United States and Europe but is much more common in Lake Maracaibo and other places where people with HD have intermarried over the generations. Will they miss out on a genetic treatment? An alternate technique is to allow both disease genes to be silenced and insert a normal gene with a mutation so that it is not recognized and silenced by the RNA interference. Dr. Patrick Aebischer and colleagues have done just that with an ALS mouse model, showing proof of principle.

RNA interference holds enormous potential for the treatment of dominant genetic disorders such as Huntington's Disease. The advances so far have been encouraging.

References

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