Delivering Solutions - UCC Researchers Focus on Gene Silencing Approaches for Huntington's Disease

More than two decades have now passed since the identification of the causative mutation for Huntington's disease (HD) by The Collaborative Huntington's Research Group, and it is now well known that HD is caused by the expression of a muHTT protein with an abnormally long polyglutamine (polyQ) tract (>40 Q) close to its N terminus.

Prof. John Cryan & Prof. Catriona O'Driscoll

In addition, an increasing body of knowledge demonstrates that the disease is caused by a toxic 'gain-of-function' mechanism rather than merely by a loss of function of the wild-type HTT (wtHTT) protein. Based on understandings of HD neuropathology, several therapeutic approaches have been advanced. These novel therapeutic modalities include neuroprotective strategies targeting the underlying pathologic mechanisms of muHTT, and cell replacement therapies focussed on counteracting neuronal loss in the brain.

However, and despite being potential alternatives to current pharmacotherapy, these strategies are aimed at downstream effects of muHTT and do not specifically target the root cause of the disease. By contrast, oligonucleotide therapeutic approaches that directly interfere with muHTT by abrogating or reducing its expression have also been considered and presented encouraging results. Among such strategies are genome-editing techniques and post-transcriptional gene silencing approaches using ribozymes and DNA enzymes, antisense oligonuceotides and RNA interference (RNAi), all of which enable a specific reduction of the synthesis of muHTT.

In fact, these approaches target upstream processes of disease and might enable therapeutic intervention even before cellular damage arises. Of these RNAi technology has emerged with great promise in areas of gene therapy development. RNAi is an endogenous cellular pathway that enables post-transcriptional regulation of gene expression. Thus when given to the appropriate site small interfereing RNAs (siRNA) can silence specific genes.

Given their potential as therapeutic strategies, lately they have received significant attention from the scientific community and the field has rapidly progressed. Reducing expression of the mutant HTT gene by means of RNA interference (RNAi) has been recently suggested as one of the most promising therapeutic strategies for HD. However, such nucleic acids have poor cell penetrating proprieties, and therefore, an appropriate delivery method is required. Moreover, for reasons not clearly understood, neurons are particularly resistant to RNAi, and therefore, delivering such molecules to the central nervous system (CNS) is very challenging. One of the primary obstacles to the progress of CNS gene-silencing technologies to the clinic is the lack of effective, nontoxic and safe delivery systems able to overcome adequately the different CNS barriers.

Some years ago two researchers in University College Cork with very different backgrounds & expertise came together to try and combine their research efforts to investigate if they could solve aspects of this puzzle. Prof. John F. Cryan is Chair of Anatomy & Neuroscience in UCC and his group has had a long-standing interesting in delivering these siRNA molecules to the brain for a variety of disorders. Prof. Caitriona O'Driscoll is Chair of Pharmaceutics in the School of Pharmacy in UCC and her lab, together with her close collaborator Dr. Rafe Darcy from UCD has focused on the design and charcaterisation of safe and effective delivery vectors for siRNA.

Together with their PhD student Bruno Godinho investigated the potential of modified sugar molecules (amphiphilic _-cyclodextrins (CDs) as novel siRNA neuronal carriers. O'Driscoll has had some success previously in using these carriers in models of cancer and inflammation but had not yet shown them to be useful for brain disorders. In work that was funded by Science Foundation Ireland via the Irish Drug Delivery Network they showed that CDs formed nanosize particles which were stable in artificial cerebrospinal fluid.

Moreover, these complexes were able to reduce the expression of the HTT gene in the laboratory in rat striatal cells (ST14A-HTT120Q) and in human HD primary fibroblasts.

Only limited toxicity was observed with CD·siRNA nanoparticles in any of the in vitro models used. Next the moved to animal models and demonstrated that there was sustained knockdown effects were observed in the striatum of the R6/2 mouse model of HD after single direct injections of CD·siRNA nanoparticles. Repeated brain injections of CD·siRNA complexes resulted in selective alleviation of motor deficits in this mouse model.

Together these data support the utility of modified _-CDs as efficient and safe siRNA delivery vectors for RNAi-based therapies for HD.

However, this approach relied on direct injections to access the brain which is far from ideal. Now in an Irish Research Council funded project O'Driscoll & Cryan and their postdoctoral fello Dr. Meena Malhotra, they are focusing on adding molecules to their carrier vectors so as to *"trick"* the blood brain barrier into allowing the entire paricles access into the brain. Such investigations are in the early days but if they can succeed could offer hope for developing siRNA-based therapies for HD. Once again such approaches would need to be shown to be effective and safe in laboratory and animal models before they could be advanced into the clinic.

For more information

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