‘Cut and paste’ gene therapy is SMarT to treat Autosomal Dominant CNM patients

Babies born with such severe muscle weakness that they struggle to breathe and swallow from the moment they enter the world, may be suffering from one of several types of centronuclear myopathy (CNM). The commonest and most severe form affecting only boys is X-linked myotubular myopathy, where the protein myotubularin is missing. Less common forms of the disease, but which can be just as debilitating, affect both sexes and include autosomal dominant forms of CNM. In this instance, the child has one normal and one defective copy of the gene. There are several different types of AD-CNM, each targeting a different gene. One type arises from mutations in the DNM2 gene encoding for the protein dynamin 2, which is important for pinching off small vesicles from the cell surface as well as regulating transport networks across the cell. However, it is far from clear how mutations in the DNM2 gene lead to muscle weakness. Even more intriguing is the majority of DNM2 mutations cause mild late-onset AD-CNM, with only a few responsible for a severe neonatal form. To understand the disease mechanism in more detail and to develop therapeutic strategies, mouse models of this disease have been created.

2011 Grant from the Myotubular Trust

The Myotubular Trust have awarded a grant to the lab of Dr Marc Bitoun, Institut de Myologie (IM), INSERM U974, Paris, France to evaluate a new gene therapy technique known as ‘spliceosome-mediated RNA trans-splicing or ‘SMarT’ for short, as a possible treatment paradigm for AD-CNM patients. Dr Bitoun has been awarded £102,340 to cover the costs of 2 years research on this project. Dr Bitoun is part of the ‘Therapy of Striated Muscle Disorders’ team based at IM, where therapies for a wide range of muscle diseases are under development. To assess the suitability of the SMarT technology to treat DNM2-AD-CNM, he will use a mouse model engineered to carry the most frequently occurring mutation in DNM2 i.e. a so-called ‘knock-in’ model.

What is SMarT ?

This is where two different RNA molecules are combined or ‘spliced’ to generate a new one. One of these RNA molecules is the one inside the patient cells encoding for the faulty gene – in this case DNM2 and the other encodes for the normal gene and is introduced into the cells using a vector. This second RNA molecule is made in the laboratory and is known as a RNA pre-transplicing molecule (PTM). When the two RNA molecules are ‘spliced’ the faulty part of the DNM2 gene in the patient’s cells is ‘cut’ out and the normal copy from the introduced vector ‘pasted’ in. The result is that the patient cells have had their faulty RNA molecule reprogrammed to encode a healthier version of the protein. The advantage of this gene therapy technique over others is that the production of the reprogrammed RNA molecule is under direct control of signalling processes associated with the patient’s cells, rather than from external factors which are less easy to control.

SMarT is the latest gene therapy technology likely to be applicable to ALL autosomal dominant diseases, not just those affecting muscle, and so has wide implications for treating genetic diseases in general.

Proposed Research

Dr Bitoun will be conducting his research in the following areas:

(i) to test the efficacy of several differently designed PTMs at repairing the faulty DNM2 gene and selecting the best PTMs to take forward. Testing will be performed in cell cultures made from the knock-in mouse.

(ii) the most efficient PTMs will be cloned into a viral vector known as adeno-associated vector which will then be injected into a leg muscle of the knock–in DNM2 mice aged 2 weeks (juvenile) or 3 months (adults).

(iii) to measure the effectiveness of the PTMs on restoring muscle functionality, both the morphology and contractile properties of the injected muscle will be examined to see if they are restored to a more normal phenotype at 1, 3 and 6 months following treatment. By treating 2 week old mice we are looking to see if we could delay the onset of symptoms, where as treating adult mice we hope to 'rescue' the phenotype.

(iv) 3 month old mice will also receive the PTMs by injection into a tail vein, so the PTMs can circulate around the body and reach all the bodies cells i.e. systemic treatment. This will allow measurement of how well SMarT works in different tissues, and how far the PTMs are able to travel from the point of injection.

If the SMarT technology is demonstrated to improve the mouse pathology of the DNM2-AD-CNM mouse, it would have a therapeutic potential to treat DNM2-AD-DNM patients carrying mutations resulting in either the severe or mild forms of the disease. Similarly, mutations in DNM2 can also cause Charcot-Marie-Tooth peripheral neuropathy (CMT), and so SMarT could be used to aid sufferers of this disease too.

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