Myotonic Muscular Dystrophy, RNA Toxicity, and the Brain: Trouble Making the Connection?

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The study of rare genetic diseases is complicated by the inaccessibility of relevant cells and tissues, especially for neurologic disorders. In this issue of *Cell Stem Cell*, Marteyn et al. (2011) use human embryonic stem cells to identify deficits in neuritic outgrowth in myotonic dystrophy type 1.

Myotonic dystrophy type 1 (DM1) is the most common inherited neuromuscular disorder in adults (Llamusi and Artero, 2008). It is also one of the most variable clinical disorders, where within one family, the most severe form—congenital DM1 (CDM1)—can affect children at birth, and the mildest form can result in only cataracts in elderly individuals. Common features of adult-onset DM1 include myotonia (prolonged muscle contractions), progressive muscle wasting, cardiac conduction abnormalities, cataracts, hypersomnolence, and various personality and executive function changes. CDM1 shows a different spectrum of symptoms, including hypotonia, mental retardation, and impaired muscle development (Meola and Sansone, 2007). DM1 is an autosomal dominant disorder caused by an expansion of a (CTG)n triplet repeat in the 3′-untranslated region of the DM protein kinase (DMPK) gene. These repeats are expanded from a normal range (n = 5 to ≈30) to greater than several thousand repeats (n = 50 to > 2000) in affected families. The size of the (CTG) expansion correlates with disease severity and tends to increase from generation to generation, explaining the variable phenotypes within one kindred. Accumulating data have established DM1 as the first example of a disorder caused by RNA toxicity (Llamusi and Artero, 2008). Mutant DMPK mRNA ("toxic RNA") aggregates into nuclear inclusions (RNA foci) and is thought to trigger dominant effects by interacting with (and altering the function of) RNA-binding proteins—primarily members of the muscleblind (MBNL) and CELF families of RNA-binding proteins—resulting in aberrant splicing of various relevant mRNAs in affected tissues.

Though neurologic manifestations are often the most pressing concern of individuals affected by DM1, little headway has been made in understanding their molecular basis. Using human embryonic stem cells (hESCs) derived from embryos with the DM1 mutation, Marteyn et al. (2011) found that after differentiation into neural precursor cells, *SLITRK4* expression was decreased, based on global expression analyses. Furthermore, the authors confirmed that expression of both *SLITRK2* and *SLITRK4* were decreased in DM1 patients, after performing RT-PCR with brain tissue samples. *SLITRK4* belongs to a newly identified family of six transmembrane proteins that exhibit homology to the Slit family of axonal growth factors and to trk neurotrophin receptors (Aruga and Mikoshiba, 2003). The SLITRK proteins are expressed primarily in neural tissues and have been implicated in affecting neurite outgrowth. Marteyn et al. (2011) differentiated the mutant hESCs into motor neurons using a coculture system with primary myoblasts and found that the resulting DM1 cells had defects characterized by increased neuritic outgrowth. Unexpectedly, this growth pattern was also associated with decreased synaptogenesis (impaired neuromuscular junction [NMJ] formation). Overexpression of *SLITRK2* and *SLITRK4* in DM1 hESCs rescued the neuritic overgrowth phenotype.

How the DM1 mutation causes these effects on *SLITRK2* and *SLITRK4* is unclear. Most pathogenesis models for DM1 have focused on RNA toxicity leading to aberrant RNA splicing (Llamusi and Artero, 2008). Alternative splicing of *SLITRK2* and *SLITRK4* has not been well studied, but the reported variants in the public databases all seem to be based on alternative 5′UTR sequences. Recently, additional effects of RNA toxicity have been reported, such as the transcriptional effects seen with *NKX2–5* (Yadava et al., 2008). *SLITRK* members may be similarly affected. Post-transcriptional effects may also be possible since CUGBP1 has been shown to play a role in RNA stability (Lee et al., 2010).

The motor neuron cell culture model employed by Marteyn et al. (2011) suggests that there are defects in motor neuron function and NMJ formation. It should be noted that the coculture experiments were done using non-DM1 myoblasts. However, in the disease state, obviously both the motor neurons and the myoblasts are affected, so it would be interesting to coculture normal and DM1 hESCs with DM1 myoblasts to study the contribution of the affected myoblasts to the observed phenotypes. This approach might also open new avenues for investigation, as muscle is a much more accessible and well-studied tissue in DM1 pathogenesis models.

In this study, the data supporting effects on *SLITRK* members in patient tissues are sparse and testify to the difficulties of studying neurologic phenotypes in rare disorders. Nevertheless, the results are intriguing in the context of DM1, where isolated studies dating back several decades have reported defects in neuromuscular junctions and hyperproliferation of noncholinergic synapses (Stranock and Davis, 1978). A recent report also identified defects in NMJs in a mouse model of DM1 expressing the toxic RNA (Panaite et al., 2008). Also, RNA foci in subsynaptic nuclei at the NMJs in muscle and in motor neurons have been found in tissues from

individuals with DM1, indicating that the toxic RNA is expressed in the relevant cells (Wheeler et al., 2007).

In childhood/juvenile-onset DM1, the major neurologic phenotypes are often related to behavioral changes, such as anxiety, attention deficit hyperactivity disorder, autistic behavior, and obsessive-compulsive behavior (Meola and Sansone, 2007). In CDM1, mental retardation is frequently observed in conjunction with the aforementioned behavioral phenotypes. In adult DM1, similar behavioral changes have been observed in many patients, including anxiety, obsessive-compulsive behavior, attention deficit disorder, and apathy. In this regard, it is of particular interest that in recent studies using knockout mice deficient for various *Slitrk* family members, a variety of behavioral phenotypes (anxiety, obsessive-compulsive behavior) and disorganized/reduced innervations have been noted (reviewed in Proenca et al., 2011). While it may be premature and speculative, the results from these knockout mice and the decreased levels of *SLITRK2* and *SLITRK4* in brains from DM1 patients support the hypothesis that the effects of the DM1 mutation on *SLITRK* members may contribute to the behavioral phenotypes observed in DM1 patients. At the least, this study provides a new target for investigating the pathology of DM1 in the brain in the many existing mouse models of RNA toxicity and highlights the potential of studies using hESCs to help unravel the pathogenesis of DM1 and other rare disorders.

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A Repair "Kit" for the Infarcted Heart

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Transplanted, c-kit expressing marrow-derived progenitors can enhance the function of an infarcted heart, but the mechanism remains unclear. In this issue of *Cell Stem Cell*, Loffredo et al. (2011) provide evidence that hematopoietic precursors do not differentiate into new cardiomyocytes but, rather, stimulate production of new cardiomyocytes from endogenous progenitors.

The stem cell biology field is no stranger to paradigm shifts, in particular when reevaluating presumed terminally differentiated tissue. As has been the case in several adult tissues, much interest over the past decade has been directed to the possibility that regenerative progenitor cells exist in the mature heart. The hearts of amphibians and teleost fish retain the ability to regenerate throughout life, and recent work in zebrafish has demonstrated this repair process occurs principally through division of pre-existing cardiomyocytes (Kikuchi et al., 2010). Interestingly, the mouse heart retains the ability to regenerate for a few days after birth, again through cardiomyocyte division, but this replication competence is lost within the first week of postnatal life (Porrello et al., 2011). While mature mammalian hearts clearly lack a robust regenerative response, mounting evidence points to some capacity for cardiomyocyte renewal. In 2007, Richard Lee's group performed a lineage tracing experiment to elegantly demonstrate that, indeed, the young adult mouse heart can generate new cardiomyocytes post myocardial infarction (Hsieh et al., 2007). Using this system, they fluorescently labeled ~80% of the pre-existing cardiomyocytes and then demonstrated that, 8 weeks post-myocardial infarction, the percentage of fluorescently labeled mature cardiomyocytes had fallen to roughly 65%, with 15% new cardiomyocytes likely arising from a progenitor population. Since this study, Frisen's group demonstrated that the adult human heart