Chemical reversal of the RNA gain of function in myotonic dystrophy

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Myotonic dystrophy type 1 (DM1) is one of a number of microsatellite expansion diseases in which the causative mutation is an aberrant expansion of a 3-nt repeat (1). The DM1 mutation was identified in 1992, and by 2001 it was established that the primary cause of pathogenesis is toxicity of the repeat-containing RNA transcribed from the expanded allele. One mechanism by which the RNA induces pathogenesis is through direct binding and sequestration of the RNA binding protein, muscleblind-like 1 (MBNL1). MBNL1 regulates a subset of alternative splicing events, and its depletion from the nucleoplasm results in misregulation of these events, causing features of the disease (2). In this issue of PNAS, Warf et al. (3) screened a relatively small number of nucleic acid binding compounds to identify those that could disrupt MBNL1–RNA interactions. The results add to the growing list of agents targeting the repeat-containing RNA of DM1 and are the first small molecules to show promising reversal of splicing defects in a DM1 mouse model.

DM1 is caused by a CTG expansion in the 3′ UTR of the *DMPK* gene. Unaffected individuals have <38 CTG repeats, whereas expansions associated with DM1 range from 80 to >2,500 repeats. Repeat length correlates directly with disease severity and inversely with the age of onset. DM1 is dominantly inherited and multisystemic; the primary causes of mortality and morbidity are progressive muscular dystrophy, cardiac-related sudden death, and CNS dysfunction (4). Therefore, systemic delivery of therapeutic compounds is a particularly attractive approach to provide benefit to the multiple organ systems affected.

The RNA expressed from the expanded *DMPK* allele (CUG^{exp} RNA) has at least two pathogenic consequences (Fig. 1). First, it forms a hairpin containing C-G base pairs and bulged U residues that binds MBNL1 with high affinity. In fact, there is a reciprocal relationship in which CUG^{exp} RNA binds and sequesters MBNL1 within nuclear foci whereas MBNL1 enhances formation of the foci and traps CUG^{exp} RNA in the nucleus (5, 6). A second component of pathogenicity is aberrant activation of protein kinase C (PKC) by

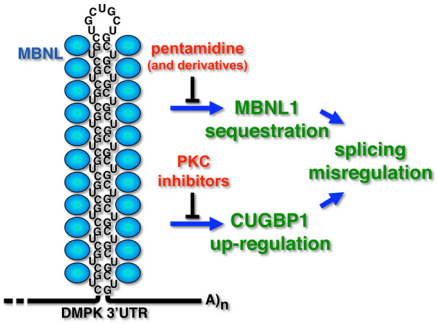

Fig. 1. Small molecules to combat the multiple toxic effects of CUG^{exp} RNA. CUG^{exp} RNA directly binds and sequesters MBNL1 and leads to up-regulation of CUGBP1 caused by PKC activation. Warf et al. (3) identified the nucleic acid binding compound, pentamidine, as an inhibitor of MBNL1-CUG^{exp} RNA interactions. PKC inhibitors have been shown to blunt the effects of CUG^{exp} RNA in a DM1 mouse model (19).

CUG^{exp} RNA that leads to hyperphosphorylation, stabilization, and up-regulation of a second RNA binding protein, CUG-binding protein 1 (CUGBP1) (7). MBNL1 and CUGBP1 are coregulators of a developmental program of alternative splicing transitions that is disrupted in DM1, resulting in misexpression of embryonic splicing patterns in adult tissues. Some features of the disease are caused by the failure of embryonic splicing patterns to fulfill the functional requirements of adult tissues (8).

The CUG^{exp} RNA provides a novel and attractive therapeutic target. Results from an inducible DM1 mouse model demonstrated that the disease phenotype improved when the pathogenic RNA was shut off, suggesting that at least some disease features are reversible (9). RNA also presents an expansive sequence and structural space that is a relatively unexplored target for small-molecule ligands. Several recent reports have applied strategic chemical design and high-throughput screening to identify compounds that block binding of MBNL1 to CUG^{exp} RNA or the $CCUG^{exp}$ RNA of the related disease, DM, type 2 (DM2) (10–13). The significance of the results of Warf et al. (3) is the logical progression from in vitro assays to testing efficacy in a DM1 mouse model. They used a stabilized CUG hairpin RNA and recombinant MBNL1 protein in a gel-shift assay to screen a small library of 26 compounds known to bind to structured RNA and found two compounds, pentamidine and neomycin B, which blocked MBNL1–RNA interactions. They then demonstrated that pentamidine (but not neomycin B) blocked the *trans*-dominant effects of CUG^{exp} RNA on splicing in cell culture by using a transient transfection assay in which CUG^{exp} RNA was coexpressed with minigene splicing reporters. Importantly, in situ hybridization to detect CUG^{exp} RNA combined with immunofluorescent staining for endogenous MBNL1 demonstrated that pentamidine caused dispersal of the RNA foci and redistribution of MBNL1 to the nucleoplasm. This finding resembles recent results demonstrating that antisense CAG morpholino oligonucleotides that block binding of MBNL1 to CUG^{exp} RNA also caused dissipation of RNA foci, MBNL1 redistribution, and down-regulation of the CUG^{exp} RNA by 50% or more (14, 15). Because morpholino oligos do not cause RNA degradation directly via the RNase H pathway, it is likely that the RNA is degraded after release from the foci. It will be of particular interest to determine whether pentamidine has this added benefit of causing loss of CUG^{exp} RNA.

The next test for efficacy was to administer pentamidine to the HSA^{LR} DM1 mouse model that expresses 250 CUG repeats within the 3′ UTR of a human skeletal α-actin transgene and reproduces DM1 splicing abnormalities (16). Pentamidine caused reversion of misregulated splicing from the embryonic toward the normal adult patterns. Although the effects of pentamidine were relatively mild in the mice, the results provide not only a proof of principle but also a chemical scaffold for strategic modifications to increase efficacy, reduce toxicity, and optimize delivery to multiple tissues.

All drugs have "off-target" effects, and in the case of pentamidine, the good news is that several alternative splicing events unaffected by CUG^{exp} RNA were not affected by pentamidine. However, the results also suggested that

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there will be a subset of MBNL1-regulated splicing events affected by MBNL1-targeted compounds. In addition, agents that target expanded RNA repeats are likely to interact with DNA. Microsatellite expansions are dynamic in that repeat number increases over the lifetime of affected individuals and this somatic instability is thought to contribute to the progressive nature of disease (17). It is therefore important to consider the possibility that compounds targeted to the RNA could also have inadvertent effects (harmful or beneficial) on the propensity of the DNA repeats to expand. Another consideration is that CUG^{exp} RNA has multiple pathological effects. Compounds that promote dissolution of the MBNL1-RNA foci and degradation of CUG^{exp} RNA should have broad beneficial effects by eliminating the primary offending agent. However, approaches that are limited to restoring MBNL1 function are unlikely to address all pathological consequences of CUG^{exp} RNA. For example, PKC activation by CUG^{exp} RNA is likely to be independent of MBNL1 depletion. Recently, administration of PKC inhibitors to a DM1 mouse model ameliorated the effects of CUG^{exp} RNA relevant to CUGBP1 up-regulation (18), suggesting that the divergent effects of the toxic RNA can be addressed by using combinatorial therapeutic approaches (Fig. 1).

Overall, the spate of recent publications supporting a wide variety of therapeutic approaches for DM1, <17 years after identification of the unusual mutation that causes disease, bodes well for the prospects of effective therapies for this and related diseases.

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