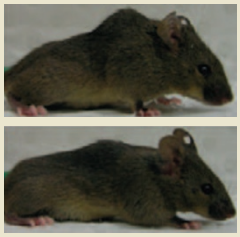
Anti-stress therapy for muscular dystrophy



Duchenne muscular dystrophy (DMD), a progressive and debilitating muscle disorder, has proved intractable to therapy, but now an approach involving a molecule already in clinical trials for a different disorder, may provide a new treatment window. Following on the observation that inflammation plays a role in disease pathology, Gehrig *et al.* targeted inflammatory mediators phosphorylated JNK, phosphorylated I-kappa-B kinase complex and tumor necrosis factor-α by expressing heat shock protein 72 (Hsp72). Using several mouse models of DMD and different methods for elevating Hsp72, the authors were able to show improvement in muscle strength and reductions in the pathological changes characteristic of the disorder. However, when they measured levels of their original inflammatory signaling targets, the researchers found these molecules were unchanged in mice overexpresssing Hsp72. This led the group to a different target for Hsp72, the sarcoplasmic/endoplasmic reticulum Ca-ATPase (SERCA), which was already known to interact with Hsp72 during times of cellular stress, as well as to be compromised in DMD patients. The researchers found increased activity of the molecule in muscle homogenates of mice overexpressing Hsp72, whereas protein and RNA levels were unchanged, suggesting that the sparing effects of Hsp72 are a result of its ability to preserve the function of SERCA. Finally, a pharmacologic inducer of Hsp72, BGP-15, which is in trials for type 2 diabetes, increased SERCA activity in dystrophic mice and improved muscle function, suggesting an immediate path to the clinic for DMD patients. (*Nature* **484**, 394–398, 2012) *LD*

Safer lentiviral vectors

Lentiviral vectors for gene therapy stably integrate into the genome of the host but also may inadvertently activate neighboring genes or disrupt splicing patterns. In an effort to design safer vectors, Cesana *et al.* identified sequences in the viral genome that influence splicing between the integrated viral genome and neighboring host genomic DNA. These sequences included known viral splice donor and acceptor sites as well as numerous splice sites described for the first time in this study. Mutating these splice sites reduced aberrant splicing, although it also reduced viral production. In addition, the authors also found more aberrant splice events when using a vector with a strong viral promoter than a vector lacking a viral promoter. Given the tendency of lentiviral vectors to integrate into actively transcribed genes, strategies to reduce interference with the host genes, such as silencing cryptic splice sites, are crucial to the development of safer vectors for gene therapy. (*J. Clin. Invest.* **122**, 1667–1676, 2012) *JK*

Protein characterization via aptamers

Approaches for assessing misfolding during therapeutic protein production are needed because such changes can expose potentially reactive epitopes, which can result in dangerous side effects. Antibodies to detect subtle protein conformation are difficult to obtain and there are not many tools to readily and routinely assess overall confirmation integrity. A group of authors from the US Food and Drug Administration show that aptamers may be able to detect minor conformational changes that are not detected by antibodies. The authors used enzyme-linked immunosorbent assays and label-free assays to assess the ability of four monoclonal antibodies and six aptamers to detect human thrombin that had been heat treated to perturb its structure without compromising its function. Whereas the panel of antibodies bound heat-treated and untreated thrombin equally well, some of the aptamers could discriminate between the two. Unlike monoclonal antibodies, which are time consuming and expensive to produce, aptamers are made using an *in vitro* selection process, greatly simplifying the production of a panel of aptamers against any protein. Aptamer panels may also be useful for assessing biosimilars. (*PLoS ONE* **7**, e31948, 2012) *JK*

Rare-cell signature predicts heart attack

No medical tests are currently available to predict when an arterial plaque is at an imminent risk of rupturing and causing a heart attack or stroke. In a retrospective human clinical study of 50 heart attack cases and 44 controls, Damani *et al.* report that the presence of elevated levels of circulating endothelial cells could be used to correctly classify 86 of the 94 patients in the study (91.5%). The circulating endothelial cells were recovered with a commercially available system that isolates rare cells using magnetic nanoparticles coated with antibodies that recognize a cell surface protein. Additional automated analysis of images from fluorescently labeled cells from eight cases and ten controls revealed that the circulating endothelial cells from heart attack patients had distinctive morphologies. Taken together, these findings are consistent with a model in which injured arteries slough endothelial cells into the bloodstream. If detected, these rare cells could serve as predictive markers of a heart attack. (*Sci. Transl. Med.* **4**, 126ra33, 2012) *CM*

Mapping the newest epigenetic mark

Methylated cytosines can be detected at single-base resolution by treating DNA with sodium bisulfite and then sequencing. Sodium bisulfite, however, does not discriminate between canonical 5-methylcytosine (5mC) and the recently discovered variant 5-hydroxymethylcytosine (5hmC), which may have unique epigenetic functions. Two groups have described methods that selectively change the chemical properties of one form of modified cytosine but not the other. Yu *et al.* protected 5hmC by enzymatic modification and then oxidized 5mC with an enzyme called TET1. In contrast, Booth *et al.* selectively oxidized 5hmC using a chemical, potassium perruthenate. In both methods, the samples were then treated with sodium bisulfite, sequenced and the resulting data were compared with data from a standard bisulfite sequencing experiment to quantitatively assess the distribution and abundance of both 5hmC and 5mC. Yu *et al.* performed a genome-wide analysis in both human and mouse embryonic stem cells, finding ~690,000 5hmC residues in human cells and ~2 million in mouse at a 5% false-discovery rate. Booth *et al.* analyzed CpG islands in mouse embryonic stem cells, finding 800 CpG islands containing 5hmC compared with ~4,500 CpG islands with 5mC at a 3.7% false-discovery rate. The studies reveal that 5hmC is enriched at enhancer elements and that 5hmC shows an asymmetric strand bias toward G-rich sequences. (*Science* **336**, 934–937, 2012; *Cell* published online, doi:10.1016/j.cell.2012.04.027, 17 May 2012) *CM*

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