Commentaries on Cutting Edge Science

Bringing It All Together
Bedside to Bench and Back Again

Jeanne James, Jeffrey Robbins

Titin Mutations in iPS Cells Define Sarcomere Insufficiency as a Cause of Dilated Cardiomyopathy
Hinson et al


A recent report illustrates the power of combining recent technological advances to define important, organ-specific phenotypes of previously refractory proteins. Genetic variants in the protein titin have been understudied because of the difficulties in handling enormous sequences and the cognate protein. But the giant protein is beginning to yield its secrets with the advent of Nex-Gen sequencing, our developing ability to create stable cardiomyocytes from patient-derived induced pluripotent stem cells (iPSCs), and the creation of 3-dimensional (3D) microtissues capable of recapitulating the stress, strain, and contractile properties of a myofibril. The ability to accurately model a patient’s pathology in isolated systems holds the promise of translating laboratory findings into effective, prospective surveillance and even therapy.

Titin is the largest characterized protein in the human body with the gene TTN consisting of 363 coding exons that theoretically could encode a 4200-kDa protein containing 38138 residues.1 Because of multiple alternative splicing events, the 3 characterized isoform classes range from ≈3000 to 3800 kDa. The location and organization of the protein are both striking. Extending some 1.0 to 1.2 μm in length, a single titin spans half the sarcomere, with the protein stretching from the sarcomere’s central M line (carboxyl terminus) to a securely anchored position in the Z disk (N-terminus) where it is closely associated with or bound to a titin molecule from the adjacent sarcomere (Figure).2 Thought to play important roles in sarcomerogenesis,3 mechanosensing mechanisms,4 and overall stabilization of the filament systems that make up the sarcomere,5 titin is an important determinant of cardiac muscle’s passive tension because of its elastic properties at low sarcomere lengths.6 The 3 TTN isoform classes found in human cardiac muscle are products of alternative splicing in the region that encodes sequences that interact with the sarcomere I- and A-bands. The isoforms are named on the basis of whether they include the different N2 domains (Figure). Thus, the ≈3000-kDa class contains only the N2B region and is called N2B titin, whereas a second larger class contains both the N2B and 2A domains and is called N2BA titin. In humans, the left ventricle has a slightly higher proportion of N2BA, whereas the atria show higher proportions of the N2B class.1 The third class contains proteins that are 3600 to 3800 kD and are highly compliant, but expression is largely restricted to the fetal/neonatal period. For additional insights into this fascinating molecule, the reader is referred to recent reviews.7,8

Against this background, one might expect that titin would be subject to intensive investigation in terms of its role in human disease, but because of its highly repetitive domain structure and sheer size, the molecule has been refractory to comprehensive analyses across the human population. However, with the advent of high throughput sequencing using the Nex-Gen technologies, sequencing an individual’s titin has become practical and titin mutations are now known to be a significant cause of cardiac disease2 with hundreds of potentially significant genetic variations identified (http://www.dmd.nl/nmdb/home.php?select_db=TTN). In a recent article, in Science, Hinson et al9 combine iPSC technology with detailed structural and functional analyses of titin mutations, using either patient-derived materials or isogenic cardiomyocytes. Three mutations were studied: 2 result in truncations in the A-band region, whereas the third produces a missense substitution at a residue located near the I/Z-disk junction.

Analyses of single cardiomyocytes derived from TTN iPSCs showed no differences in contractile function compared with cardiomyocytes derived from a control. Recognizing that single cells do not accurately recapitulate native cardiomyocyte architecture or protein complement, the authors turned to tissue engineering to create 3D cardiac microtissues (CMTs) embedded in a 3D micropatterned matrix.10 CMTs containing the truncation mutations or the missense mutation displayed reductions of >50% in contractile force, confirming both the efficacy of the system and the pathogenic nature of the particular mutations. The effects of background genetic variation were subsequently studied by engineering the mutations into isogenic iPSCs and repeating the CMT assays. Although reductions in contractile force persisted in the isogenic-derived CMTs, the magnitude of change was smaller, suggesting that genetic background contributes to the overall pathogenicity of a TTN mutation.

Hinson et al9 then used their ability to sequence titin transcripts to explore the apparent differences in penetrance and pathology among mutations present in the regions that overlap and interact with the sarcomere A- and I-bands. Although
A-band exon truncation mutations invariably lead to a discernible pathogenic phenotype, many healthy individuals are known to carry nonsense mutations in I-encoding exons. Sequence analyses indicated that the transcript exons corresponding to the I-band sequences were sometimes spliced out of the mature transcript, resulting in a sufficient amount (18%) of titin transcript lacking the mutated exon. The 18% of normal titin may well be sufficient to prevent an overt phenotype from presenting. Taken together, the present data argue for a hypothesis that these alternative splicing events, in which the nonsense mutation–containing exon is removed, account for the reduced penetrance of I-band truncation mutations.

In light of the above data on I-band truncations, the authors assessed the effect of A-band truncations in a similar fashion. They found that, in addition to decreased contractile force, iPS cardiomyocytes heterozygous for an A-band truncation displayed significant reductions in myofibrils with sarcomeres that were both abnormal in appearance and reduced in number. These findings were even more pronounced in the homozygous cells. RNA sequencing and protein analyses indicated that for some A-band truncations, the truncated transcript was stable and normal amounts of the protein were present, indicating that the mutated form was unable to support normal sarcomerogenesis and maintenance. Considering the mechanical deficits, the authors went on to show limited contractile reserve in response to β-adrenergic stimulation. Attenuated activation of normal growth factors and cell signaling molecules was present as well. They concluded that the titin A-band truncations cause dilated cardiomyopathy as a result of sarcomere insufficiency and the resultant disruption of linkages between sarcomere biogenesis, function, and adaptive remodeling.

How might this seminal article on patient-derived iPSCs affect the diagnosis and care of patients with genetically induced cardiomyopathy? In 2006, Takahashi et al.11 first reported the generation of pluripotent stem cells from fibroblasts. This catalyzed a series of experiments leading to the development of techniques capable of differentiating iPSCs into multiple cell lineages. The excitement surrounding these early investigations with respect to the potential for developing patient-specific iPSCs was clearly warranted. Expression of human mutations in nonsyngenic species by transgenic and gene-targeting approaches has produced important knowledge, but the intrinsic biological differences between mice and humans are significant limitations to these studies.

Patient-derived iPSCs facilitate the determination of the functional consequences of a particular mutation. Although the linkage between these particular titin mutations and dilated cardiomyopathy was already persuasive, in many situations, it is difficult to assign causality to sequence variations revealed by genetic testing. Designation of a sequence variation as a truly disease-causing mutation and not a variant of unknown significance occurs if the variation segregates with a detectable phenotype in an extended pedigree or if predictive algorithms suggest a substantial alteration in critical structural motifs.12 Large proteins, such as titin, are particularly difficult to assess in this fashion because of complexities in the molecular structure. The demonstration of functional changes in reagents derived from iPScs can, thus, provide supportive evidence of a true mutation, potentially accelerate the determination of causality, and mitigate the need to identify, locate, and screen all at-risk family members for what might be only a rare polymorphism.

Unlike biopsy specimens, iPSCs are a renewable source of patient-derived materials. iPSCs provide investigators a more flexible system with which to determine whether drugs that seem clinically beneficial in one cell lineage have potentially undesirable effects in other cell types. The ability to rapidly develop or screen favorable compounds while promptly abandoning ineffective or dangerous biologicals has the potential to dramatically decrease the time required for new drug discovery. Furthermore, the ability to print large arrays of cells and CMTs using bioengineering techniques16 indicates the paradigm established by Hinson et al10 is scalable, and it will eventually be possible to conduct high throughput drug screens on a patient by patient basis.

Besides determining the phenotypic consequences of a particular mutation, patient-specific iPSCs will be valuable tools for exploring the role of genetic modifiers. Hypertrophic cardiomyopathy is a classic example of an autosomal dominant disease with variable penetrance and expressivity. Phenotypic variability is commonly ascribed to the presence of genetic modifiers that confer either susceptibility to or protection from the development of cardiomyopathy. To date, only a handful of such modifiers have been unequivocally identified.11 Consider the value of being able to develop a testing algorithm to determine whether drugs that target specific iPSCs demonstrates resistance or susceptibility to disease, although this in itself would be significant information. Likewise, the application of genomic, RNA sequencing and proteomic tools to reagents derived from members of informative pedigrees will likely accelerate the discovery of such modifiers.

**Figure.** Titin in the sarcomere. **Top,** An electron micrograph of a normal sarcomere. **Bottom,** A schematic diagram of the 3 major filament systems: the thick filament containing myosin, the thin filament containing actin, and the titin filament. The orientation and relative positions of the amino and carboxyl titin termini and regions corresponding to the A- and I-bands are shown. The locations of the N2B and N2A regions, which are used to distinguish the different titin protein classes (see text), are shown. At the bottom of the figure, 2 titin molecules spanning the entire sarcomere are shown with the thick and thin filaments removed for clarity.
Of course, all new technologies have drawbacks. As reported by Hinson et al, merely differentiating patient-derived iPSCs into cardiomyocytes did not result in an obvious phenotype. Only when the cells were processed into CMTs, the functional consequences of titin truncating variants were apparent. Cardiomyocytes derived from iPSCs do not fully recapitulate the mature native cell in terms of individual cellular architecture and differential gene expression. Furthermore, the heart is vastly more than the sum of its individual cardiomyocytes. One cannot extrapolate to the whole animal discoveries made in cultured cells, which by design lack normal cell–cell contact, electric impulse conduction, and neurohormonal influences. Although reproducing the true intracellular and intercellular milieu is presently a challenge, increasingly sophisticated experimental designs and advances in tissue engineering scaffolding suggest that the field has not yet reached its limits.

At this point, using iPSCs for clinical investigations remains time and resource intensive and is largely restricted to major academic biomedical centers. However, one may reasonably expect that these techniques will rapidly become commoditized and more scalable. Furthermore, in recent years, there has been considerable media discussion on the ethics of stem cell research. Although such public conversations often seem misguided to the scientific community, to ensure widespread acceptance of iPSC technology and the support of extramural funding agencies, the general public must gain a basic understanding of stem cell technology, particularly the use of fibroblasts to develop the iPSCs.

A Potent Mix of Technique and Approach

Hinson et al have demonstrated the potential of marshaling a series of techniques to materially move the field forward. Although all the requisite techniques (ie, iPSC production, RNA sequencing, and 3D CMT) have become available in the recent past, their assembly and use in a well-described experimental outline are important and novel. Like many elegant demonstrations, its conception in retrospect seems obvious, but the authors have illustrated its power to provide mechanistic insight into patient phenotypes. A further contribution is an introductory understanding of the basis for variable penetrance of different mutations within a single protein. Furthermore, the use of CMTs points to the importance of 3D systems in exploring contractile and hemodynamic deficits in cardiomyopathy and highlights the limitations of single-cell preparations.

With this report, harnessing the power of patient-derived iPSCs to deliver truly patient-specific therapies becomes more than a theoretical strategy. The ability to study the effects of an individual’s genetic mutation within the context of his or her own genetic modifier background has the potential to dramatically accelerate treatment modifications. Already extensively used in cancer treatment because of the ready availability of tumor cells, molecular profiling will become more than a theoretical approach for designing patient-specific treatment of cardiac disease. Undesirable or unexpected by-stander effects may be discovered in the laboratory, mitigating patient risk and accelerating treatment for those who have failed to respond to conventional treatments. Bedside to bench and back again is the ultimate goal of individualized medicine, and Hinson et al have brought it closer to practice.

Sources of Funding

This work was supported by National Institutes of Health grants P01HL69779, P01HL059408, and R01HL105924 and the Transatlantic Network of Excellence Program grant from Le Fondation Leducq (to J. Robbins).

Disclosures

None.

References
