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Skin Deep: What Can the Study of Dermal Fibroblasts Teach Us About Dilated Cardiomyopathy?

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Human heart tissue is difficult to obtain. Its procurement depends upon access to a center at which heart transplants or ventricular assist device implantations occur, as well as a willingness to serve science at odd hours and on short notice. As if these barriers weren't sufficiently discouraging to the would-be translational investigator, there are specific and thorny challenges associated with isolating, culturing, and studying human cardiomyocytes. Even observations made in meticulously procured human myocardium and cardiomyocytes are subject to limitations, as outlined in a thoughtful review published previously in this journal. [1] Thus the cardiovascular research community relies largely upon animal models to study myocardial disease, the most common of which is the genetically altered mouse. Such models often do predict human biology accurately, however testing their inherent assumptions is undeniably important and remains central to the biomedical research enterprise. In this regard, the availability of human heart tissue stands as one significant rate-limiting step in faithful translation, and novel approaches to the investigation of human myocardial biology are needed.

Familial dilated cardiomyopathy (DCM) seems particularly well suited to the use of genetically altered mouse models for elucidation of cellular mechanism, both because the disorder almost always arises from a single mutation, and because of the aforementioned difficulties in obtaining cardiomyocytes from the human proband. Once thought to be rare, familial DCM now appears to constitute 20–50% of all idiopathic DCM,[2] which is the underlying diagnosis in 50% of all heart transplants. [3] More than twenty genes have been implicated in familial DCM,[4] the majority of which encode sarcomeric or cytoskeletal proteins that are involved in the generation or transmission of force. [5] That is to say that on a cellular level, familial DCM has been understood largely as a disease of force-generating cells, and investigation of the cellular biology of this disorder has focused almost entirely on the cardiomyocyte, that uniquely cardiac cell type.

This paradigm received its first stiff challenge roughly a decade ago, when Fatkin et al identified numerous cases of DCM associated with mutations in the gene that encodes the nuclear envelope proteins lamin A and lamin C. [6] Since then, over 300 mutations in *LMNA* have been described, resulting in phenotypes that range from lipodystrophy to progeria, [7]

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but the majority lead to some form of cardiomyopathy. [8] In fact, mutations in *LMNA* are now understood to be among the most common causes of familial DCM. [9] The cellular mechanisms that underlie the cardiomyopathies remain incompletely defined, and it is not entirely clear why the heart is so disproportionately affected by mutations in this ubiquitously expressed gene. These questions are begged by the lamins' promiscuity: in addition to anchoring the nuclear membrane, lamins exist in the nucleoplasm and have been shown to interact with transcription factors, DNA, and chromatin, [10] and may be involved in nuclear signaling. [11] It is assumed that cardiomyopathy results from abnormally functioning lamins in cardiomyocytes -- cardiomyocytes isolated from *LMNA*-null mice do exhibit contractile dysfunction--[12] however other cell types clearly are affected.

Lamins are expressed in nearly all differentiated cell types. In contrast to many of the genes presently known to cause familial DCM, *LMNA* mutations do not require the presence of a sarcomere to perturb cellular function. It is assumed, however, that the resultant defects are present in both myocytes and non-myocytes alike. Thus it may be feasible to use human cells to study the human cell biology of human cardiomyopathy, without having to isolate human cardiomyocytes. Multiple groups have seized upon this opportunity, making clever use of dermal fibroblasts from patients with cardiomyopathies to elucidate mechanisms of cellular dysfunction. Muchir et al identified structural abnormalities in the nuclear membrane of such fibroblasts; [13] Emerson et al recently found abnormal cell spreading and dysregulated ERK 1/2 phosphorylation; [14] and Zhang et al showed that sumoylation regulates normal subcellular localization of lamin A. [15] This approach also has been applied to the investigation of nesprins, nuclear envelope proteins that connect the actin cytoskeleton to the nuclear lamins.[16] Mutations in the genes encoding the nesprins were identified in patients with DCM due to Emery-Dreifus muscular dystrophy (EDMD). Dermal fibroblasts from these patients demonstrate dysmorphic nuclei and impaired interactions within the LINC (Linker of Nucleoskeleton and Cytoskeleton) complex, [17] which consists of the nesprins, emerin, lamin, and the SUN proteins. [16]

In this issue of the *Journal of Molecular and Cellular Cardiology*, Puckelwartz et al demonstrate that mutations in nesprin-1a also can lead to DCM with phenotypes that resemble those of the isolated cardiomyopathies due to *LMNA* mutations. [18] The authors screened for nesprin-1a mutations in 46 unrelated individuals with DCM and identified a single variant (R374H) that localized to a region that is known to bind lamin A. The carrier of this mutation was a young man with a family history of heart failure who had undergone heart transplantation for severe DCM. To further investigate the etiologic role of nesprin-1a mutations in cardiomyopathy, the authors studied mice with a homozygous disruption of a Klarsicht, ANC-1, and Syne Homology (KASH) domain in nesprin-1 that interacts with SUN2 in the perinuclear space. This genetic alteration leads to dysfunction of the LINC complex, and the D/D KASH mouse has a phenotype consistent with EDMD. [19]

To assess the cellular effects of the R374H variant in the proband, the authors studied fibroblasts derived from his skin. Immunoblots using these cells were compared with immunoblots of D/D KASH heart lysates, and both revealed alterations in the LINC complex. Surface electrocardiograms and intracardiac electrophysiologic recordings of D/D KASH mice demonstrated atrial and ventricular conduction defects and echocardiography revealed impaired systolic function. The combination of conduction system disease and systolic dysfunction is noteworthy as it bears a striking resemblance to the well-recognized cardiac manifestations of laminopathies. [6,20] This similarity was substantiated by the authors' findings that the nuclei of Δ/Δ KASH cardiomyocytes have structural abnormalities and a paucity of heterochromatin, as have been observed in myocytes from both mice and humans carrying *LMNA* mutations. [21,22] The authors conclude that mutations in nesprin-1a cause similar cardiac defects as mutations in *LMNA*, and that those defects may

be mediated through alterations in the inner nuclear membrane that arise from abnormal structural interactions, or through aberrant signaling.

This exciting report does leave room for further investigation. The nesprin-1a mutation in the human proband differed from that in the mouse model, and the comparison would have been enriched by further phenotypic details from the proband's medical history. In the absence of such data, an expanded focus on the biology of the proband's dermal fibroblasts could have been illuminating. Nevertheless, the authors have made important discoveries that expand our understanding of the role of the nuclear envelope in the pathobiology of familial DCM. The paper is noteworthy not only for its findings, but for its methods, insofar as both a genetically altered mouse model and a human non-myocyte cell type were used to advance our understanding of human heart muscle disease.

Despite much progress, the etiology of a substantial number of cardiomyopathies remains "idiopathic". However if recent trends hold, it is anticipated that a genetic etiology will be identified for a substantial portion of these idiopathic DCM cases. As detection and sequencing techniques become ever more powerful, the pace of this discovery will quicken. Assessing the functional significance of these newly identified mutations will require experimental platforms with the capability for higher throughput than the de novo creation of genetically altered mouse models. For initial analysis of putative disease-causing mutations in broadly expressed genes, the use of cultured human dermal fibroblasts holds some promise. The identification of provocative cellular abnormalities in a proband's fibroblasts could then justify the time and expense required to create a relevant knockout mouse, and more direct investigation of myocardial biology.

The observation of abnormalities in dermal fibroblasts from patients with cardiomyopathies also opens avenues for further speculation regarding the pathobiology of familial DCM. Perhaps our focus on the cardiomyocyte has been not only arduous, but myopic. It is becoming increasingly clear that dysfunction of non-myocytes—to include cardiac fibroblasts and endothelial cells—not only results from, but contributes to the progressive failure of the heart muscle. Perhaps there is a subset of the as yet idiopathic DCMs in which non-myocytes are the site of the primary abnormality, rather than merely bystanders to the malfunction of the myocyte. Indeed, a recent study hinted at that possibility, employing both zebrafish models and human endothelial cell culture to demonstrate that mutations in the laminin and integrin system cause DCM by concomitant primary dysfunction of both endothelial cells and cardiomyocytes. [23]

Clearly, the use of human dermal fibroblasts does not obviate the need for ongoing investigations in both human heart tissue and animal models. Fibroblasts are not cardiomyocytes, and thus are ill suited for studying many aspects of myocardial biology. However for the appropriately chosen question, such as the one addressed by Puckelwartz et al, they can provide valuable insight into mechanisms of cellular dysfunction. And because fibroblasts are not cardiomyocytes, they can be obtained without the need for highly specialized surgical services (or further sleep deprivation for research personnel) and can be maintained in culture indefinitely. There is much still to be learned about the inner workings of the heart muscle and some of the rest may be gathered by remaining skin deep.

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