For over a hundred years, the fruit fly, *Drosophila melanogaster*, has provided wonderful insights into developmental biology and the signaling molecules that regulate tissue growth and homeostasis. The wealth of mutants, rapid breeding times, and robust resources make *Drosophila* an ideal genetic model. The development of the fly cardiac system follows a series of orchestrated events and involves transcription factors and molecules conserved among species, including humans. More recently, the fly has been used to model human heart diseases and better understand cardiomyopathies.

Because nephrocytes are endocytic and clear extracellular proteins, it was hypothesized that *dklf15* NN/NN mutants would have enrichment of specific extracellular proteins and these factors may be responsible for the cardiomyopathy observed in the absence of pericardial nephrocytes. To address this hypothesis, proteomic analyses were conducted to identify peptides that were enriched in the hemolymph of *dklf15* NN/NN. Additional filtering algorithms identified proteins that had predicted signal peptides consistent with extracellular proteins. Among the peptides that had increased abundances in the absence of nephrocytes, secreted protein acidic and rich in cysteine (SPARC) was highly enriched and identified as a potential candidate responsible for the nonautonomous changes in cardiac function (Figure).

SPARC, also known as Osteonectin or BM-40, is an evolutionarily conserved matricellular protein composed of an acidic amino terminal domain, a Follistatin-like domain, and a high-affinity calcium-binding EF hand carboxyl terminal domain.10–11 Expressed during many stages of development in *Drosophila*, *Caenorhabditis elegans*, mice, and humans, SPARC expression is restricted in adult vertebrates primarily to tissues that undergo consistent turnover or to sites of injury and disease.12–16 SPARC functions through several proposed mechanisms including, but not limited to, modulation of (1) matrix metalloproteinases, critical mediators of extracellular matrix proteolysis and turnover; (2) growth factor signaling mediated by cell surface receptors, including vascular endothelial growth factor receptor, basic fibroblast growth factor, and transforming growth factor-β1; (3) integrin and Integrin-Linked Kinase activity; and (4) monocyte/macrophage recruitment.12–16

Studies of transgenic mice have shown that the deletion of SPARC alters the balance between processing to mature collagen fibrils and degradation.17 This lack of SPARC leads to increased cell-associated collagen degradation or uptake by cell surface collagen receptors resulting in less procollagen effectively processed into mature fibrils and decreased levels of interstitial collagen assembled into insoluble fibers.13 Conversely, SPARC overexpression promotes efficient procollagen processing and incorporation of collagen into fibrils thus enhancing deposition of collagen and formation of mature fibers.

In response to transverse aortic constriction, wild-type mouse hearts exhibit enhanced collagen deposition, augmented SPARC expression, and increased myocardial stiffness.18 Interestingly, the hearts from global SPARC knockout mice that underwent transverse aortic constriction had decreased collagen deposition and myocardial stiffness.18 After myocardial infarction, SPARC mRNA and protein levels increased in the infarct and remote zones—paralleling collagen deposition.19 SPARC knockout mice had higher rates of myocardial rupture and ventricular dysfunction after myocardial infarction and the overexpression of SPARC by adenoviral delivery to wild-type mice improved cardiac function.
after myocardial infarction. Interestingly, SPARC has also been implicated in the modulation of transforming growth factor-β1 and myofibroblast conversion, again suggesting additional roles of SPARC in the maintenance of myocardial function after injury.19

The observation that the levels of SPARC and other proteins were increased in dKlf15NN/NN mutants, lacking nephrocytes, has significant implications. Importantly, questions about the mechanisms that underlie the observations by Hartley et al9 require further investigations.

First, how does SPARC induce the cardiac abnormalities in the dKlf1/5NN/NN mutants and how does SPARC function in normal cardiac function in Drosophila? In Drosophila embryos, SPARC is synthesized and secreted by hemocytes, promoting collagen IV localization at the basal lamina.20 Moreover, SPARC is essential for collagen IV assembly into the basal lamina and for its proper secretion from fat body cells. In mammals, SPARC functions as a matricellular protein, namely an extracellular matrix–associated protein that does not serve the classical structural roles of laminins and collagens. As mentioned, in response to transverse aortic constriction, wild-type mouse hearts exhibit increased collagen deposition, SPARC expression, and myocardial stiffness.18 The hearts from global SPARC knockout mice that underwent transverse aortic constriction had decreased collagen deposition and myocardial stiffness. The cardiac abnormality in dKlf15NN/NN mutants observed by Hartley et al was mainly an increase in the diastolic period. Assuming that a lengthening in the diastolic period is a surrogate for diastolic function in flies, the increased levels of SPARC in the dKlf15NN/NN may represent changes in myocardial stiffness in the Drosophila heart. Perhaps, the fly model may lead to better understanding of the pathways that contribute to diastolic dysfunction and heart failure in humans.

Second, are there additional mechanisms through which SPARC is acting in the Drosophila heart that are not the direct result of changes in the extracellular matrix? Cells compete for survival during organ growth, homeostasis, and aging/senescence. In Drosophila, SPARC was shown to be transcriptionally upregulated early in loser cells to provide transient protection by inhibiting caspase activation in outcompeted cells.21 Interestingly, the silencing of SPARC in cardiomyocytes using tinD-Gal4 caused an increase in end-systolic stretch.

Figure. Secreted protein acidic and rich in cysteine (SPARC)–mediated cardiac dysfunction in flies. In the wild-type, SPARC and other factors are taken up and presumably degraded by the endocytic pericardial nephrocytes. dKlf15NN mutant flies lack pericardial nephrocytes resulting in an accumulation of SPARC and other factors in the extracellular space that cause cardiomyocyte dysfunction. ECM indicates extracellular matrix.
dimension and reduction in fractional shortening, suggesting additional mechanisms through which SPARC mediates influence on cardiac function. Could alterations in SPARC levels change other ligands, receptor expression on the surface of cardiomyocytes, or the metabolic profile of cardiomyocytes that cause the abnormalities in cardiac function observed in dKlf15NN flies? Could these mechanisms contribute to SPARC-mediated changes in heart function?

Third, are there factors expressed by pericardial cells that are lost in the dKlf15NN flies that also contribute to the phenotype? If so, are these conserved across species—including humans? Can the identification of these molecules provide insight into cardiomyocyte health and cardiac function? The robust data sets generated by Hartley et al provide a framework to address these important questions.

The findings reported by Hartley et al further highlight the growing interest in Drosophila as a model system of cardiovascular diseases. Furthermore, the studies serve as an example of the conservation of signaling molecules among species and the power of comparing the interspecies differences to identify the signals that govern cardiac biology.

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References
1. Bodmer R, Venkatesh TV. Heart development in Drosophila and vertebrates: conservation of molecular mechanisms. Dev Genet. 1998;22:181–186.
2. Bier E, Bodmer R. Drosophila, an emerging model for cardiac disease. Gene. 2004;342:1–11. doi: 10.1016/j.gene.2004.07.018.
3. Diop SB, Bodmer R. Gaining insights into diabetic cardiomyopathy from Drosophila. Trends Endocrinol Metab. 2015;26:618–627. doi: 10.1016/j.tem.2015.09.009.
4. Wolf MJ. Modeling dilated cardiomyopathies in Drosophila. Trends Cardiovasc Med. 2012;22:55–61. doi: 10.1016/j.tcm.2012.06.012.
5. Wolf MJ, Rockman HA. Drosophila, genetic screens, and cardiac function. Circ Res. 2011;109:794–806. doi: 10.1161/CIRCRESAHA.111.244897.
6. Miller, A. The internal Anatomy and Histology of the Imago of Drosophila melanogaster: The Biology of Drosophila (Demerec, M. ed.) Plainview, NY: Cold Spring Harbor Laboratory Press; 1994.
7. Weavers H, Prieto-Sánchez S, Grawe F, García-López A, Artero R, Wilsch-Bräuninger M, et al. The insect nephrocyte is a podocyte-like cell with a filtration slit diaphragm. Nature. 2009;457:322–326. doi: 10.1038/nature07526.
8. Iyer JR, Drechsler M, Catterson JH, Bodmer R, Ocorr K, Paululat A, et al. Klf15 is critical for the development and differentiation of Drosophila Nephrocytes. PLoS One. 2015;10:e0134620. doi: 10.1371/journal.pone.0134620.
9. Hartley PS, Motamedchaboki K, Bodmer R, Ocorr K. Secreted protein acidic and rich in cysteine–dependent cardiomyopathy in Drosophila. Circ Cardiovasc Genet. 2016;9:119–129. doi: 10.1161/CIRCGENETICS.115.001254.
10. Bradshaw AD, Sage EH. SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury. J Clin Invest. 2001;107:1049–1054. doi: 10.1172/JCI12939.
11. Brekken RA, Sage EH. SPARC, a matricellular protein: at the crossroads of cell-matrix. Matrix Biol. 2000;19:569–580.
12. Tremble PM, Lane TF, Sage EH, Werb Z. SPARC, a secreted protein associated with morphogenesis and tissue remodeling, induces expression of metalloproteinases in fibroblasts through a novel extracellular matrix-dependent pathway. J Cell Biol. 1993;121:1433–1444.
13. Bradshaw AD. The role of secreted protein acidic and rich in cysteine (SPARC) in cardiac repair and fibrosis: does expression of SPARC by macrophages influence outcomes? J Mol Cell Cardiol. 2015; doi: 10.1016/j.yjmcc.2015.11.014.
14. Kupprion C, Motamed K, Sage EH. SPARC (BM-40, osteonectin) inhibits the mitogenic effect of vascular endothelial growth factor on microvascular endothelial cells. J Biol Chem. 1998;273:29635–29640.
15. Nozaki M, Sakurai E, Raisler BJ, Baffi JZ, Witta J, Ogura Y, et al. Loss of SPARC-mediated VEGFR-1 suppression after injury reveals a novel antiangiogenic activity of VEGF-A. J Clin Invest. 2006;116:422–429. doi: 10.1172/JCI26316.
16. Rivera LB, Brekken RA. SPARC promotes pericyte recruitment via inhibition of endoglin-dependent TGF-β1 activity. J Cell Biol. 2011;193:1305–1319. doi: 10.1083/jcb.201011143.
17. Bradshaw AD, Biauc C, Rentz TJ, Van Laer AO, Bonnema DD, Zile MR. Age-dependent alterations in fibrillar collagen content and myocardial diastolic function: role of SPARC in post-synthetic procollagen processing. Am J Physiol Heart Circ Physiol. 2010;298:H614–H622. doi: 10.1152/ajpheart.00474.2009.
18. Bradshaw AD, Biauc CF, Rentz TJ, Van Laer AO, Boggs J, Lacy JM, et al. Pressure overload-induced alterations in fibrillar collagen content and myocardial diastolic function: role of secreted protein acidic and rich in cysteine (SPARC) in post-synthetic procollagen processing. Circulation. 2009;119:269–280. doi: 10.1161/CIRCULATIONAHA.108.773424.
19. Schellings MW, Vanhoutte D, Swinnen M, Cleutjens JP, Debets J, van Leeuwen RE, et al. Absence of SPARC results in increased cardiac rupture and dysfunction after acute myocardial infarction. J Exp Med. 2009;206:113–123. doi: 10.1084/jem.20081244.
20. Volk T, Wang S, Rotstein B, Paululat A. Matricellular proteins in development: perspectives from the Drosophila heart. Matrix Biol. 2014;37:162–166. doi: 10.1016/j.matbio.2014.03.006.
21. Portela M, Casas-Tinto S, Rhiner C, López-Gay JM, Domínguez O, Soldini D, et al. Drosophila SPARC is a self-protective signal expressed by loser cells during cell competition. Dev Cell. 2010;19:562–573. doi: 10.1016/j.devcel.2010.09.004.

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