Developing Hearts Need Their SPEG

Mark A. Sussman, PhD

Myocardial formation is a particularly bad time to be missing building blocks and tools essential for myofibrillogenesis. Prenatal development and early postnatal development are characterized by intense cardiomyocyte formation and contractile apparatus assembly critical for normal growth and maturation. In this rapidly evolving milieu of cardiac formation, disturbances of cardiomyocyte organization or contractile performance usually have consequences leading to cardiomyopathic damage or lethality.\textsuperscript{1,2} Sensitivity of the developing heart to perturbation makes it a fertile playground for genetic engineering to demonstrate phenotypic effects resulting from assembling myocardium with missing or malfunctioning molecular pieces. Deliberate DNA recombination mischief with a knock-in mouse described by Liu et al\textsuperscript{3} now finds another way to jumble the puzzle by disruption of the striated preferentially expressed gene (SPEG). Loss of SPEG results in a postnatal dilated cardiomyopathic phenotype, combined with neonatal lethality, that indicates that appropriate myocardial development requires SPEG participation. These findings raise questions regarding what role SPEG fills in the construction of healthy myocardium and whether alterations in SPEG are linked to cardiomyopathic remodeling in the mature heart.

SPEG is a member of a family of myosin light-chain kinases (MLCKs) that are required for cytoskeletal remodeling in myocytes. The MLCK domains of family members trace their evolutionary origins from mammals all the way back to Unc-89, an obscurin-MLCK found in Caenorhabditis elegans.\textsuperscript{4} Certain MLCK members are implicated in the regulation of the actin-based cytoskeleton and sarcomeric reorganization via phosphorylation of downstream targets such as myosin light-chain 2V.\textsuperscript{5} As the name implies, SPEG is expressed vin striated muscle, leading to the presumption that it plays a critical role in the regulation of the myofibrillar structures found therein. Because SPEG is induced during myogenesis but is not present in myoblasts, a role in myofibril formation during differentiation seems likely, especially since SPEG is localized to the Z disk that is home to a host of molecules that direct myofibril assembly and maturation of myofibrils.\textsuperscript{6}

Disturbance of SPEG locus in the engineered mice was made possible by replacement of 3 commonly shared exons with a β-galactosidase coding sequence. Reporter gene activity was detected in the hearts of the knock-in mice at day 11 in utero. This early expression profile for SPEG likely accounts for the dramatically impaired myocardial development observed in developing hearts of these mice, which began enlarging before birth and showed significant neonatal mortality within days after birth. Because there were no hallmarks of structural abnormalities such as septal defects or valvular abnormalities, the blame for loss of function lies primarily with the cardiomyocytes themselves. Hearts subjected to phenotypic assessments showed typical signs of disrupted cardiomyocyte maturation characterized by myofibril degeneration, dilatation, and impaired functional performance. Coupled with the absence of a marked increase in apoptotic cardiomyocyte death, the problem seems to be contractility of the cells themselves, not a loss of cell number. The underlying basis of altered contractility in the cardiomyocytes could stem from a number of possibilities, but the connection of SPEG to the MLCK family led Liu et al to examine the phosphorylation status of tropomyosin, which was found to be altered in the failing SPEG hearts relative to normal controls.

So, what can we derive from these first forays into the cardiac biology of SPEG? As with most initial characterizations, much work remains to be done, and many questions remain unanswered. Although Liu et al point to the phosphorylation of tropomyosin as a causal factor in the pathogenesis of “SPEG-lessness,” it is impossible to ascertain whether the altered phosphorylation observed resulted from impaired SPEG function or the ensuing posttranslational consequences of cardiomyopathic remodeling for many sarcomeric proteins. SPEG may provide critical regulatory control of prenatal myocardial development, as shown in the Liu et al study, but little change in SPEG occurs in response to cardiac hypertrophy and dilated cardiomyopathy.\textsuperscript{7} Thus, it remains to be determined whether the loss or alteration of SPEG in the mature myocardium has any profound impact on contractile function and myofibril organization. Functional signaling redundancy, together with the establishment of mature organized myofibrils, likely renders mature myocardium less sensitive to altered SPEG function than observed in the milieu of prenatal growth, so it is also premature to point to alteration of SPEG as a basis for hereditary cardiomyopathies in human populations until careful linkage analyses and identification of candidate genes can be confirmed. Last but not least, although the role of SPEG in responses and adaptations to cardiomyopathic stress or injury in the mature remains unclear, there may be opportunities to explore this possibility using the heterozygous SPEG\textsuperscript{−/−} mice with hearts that appear structurally normal but show diminished levels of


protein expression compared with wild-type littermates. Presence of the β-galactosidase reporter gene in the SPEG−/− mice also provides an interesting readout for the distribution and expression level of SPEG throughout the body.

SPEG α and β isoforms were previously reported to be expressed in the heart only after birth, whereas Liu et al. assert that these isoforms were the only ones present in cardiomyocytes of the prenatal heart. In fact, the SPEG locus codes for multiple family members, including APEG-1 and BPEG-1, which are found preferentially in aortic and brain tissues, respectively. Message transcripts for both APEG and BPEG are absent in the homozygous SPEG−/− mice, so subtle effects resulting from the deletion of these isoforms could also confound straightforward interpretation of the results. Indeed, strong APEG-1 expression is observed in late fetal and early postnatal development in the elastic arteries, including the aorta, carotid, and pulmonary vessels. Teasing out the cause-and-effect relationships in this system requires additional studies.

The 2 tandem MLCK motifs found in SPEG proteins also are present in a related subfamily known as the obscurins. Recent studies suggest that SPEG arose from the kinase domains of obscurin-MLCK via a gene duplication event and that the 2 families of proteins have overlapping phosphorylation targets. Other regions of these molecules possess domains responsible for anchoring the kinase to specific intracellular sites and enable interaction with specific target molecules. Like the SPEG family, obscurin has a critical role in myofibrillogenesis and early cardiac development. Parsing out relationships between the SPEG and obscurin families is currently in elementary stages, with implications for roles of these proteins in myofibril organization and the cardiac response to remodeling.

At the end of the saga, it becomes apparent that the SPEG/obscurin family of proteins are important players in early cardiac development, likely because of essential roles in the formation and alignment of nascent myofibrils. Consequently, the impairment of SPEG in prenatal development leads to compromised contractility that subsequently transitions into the constellation of features observed by Liu et al: lousy-looking myofibrils, enlarged unhappy cardiomyocytes, big compensated hearts, miserable cardiac contractility, and a great deal of early postnatal lethality. Because the prenatal heart is hypersensitive to structural disruption compared with the adult myocardium, it remains to be seen how essential the SPEG proteins are in the ongoing compensation of the mature myocardium. In addition, it is premature to pin the phenotypic effects from the knock-in on a single mechanistic explanation of altered tropomyosin phosphorylation. Although current studies have left many questions unanswered, there is one thing we can look forward to: More details will undoubtedly be forthcoming as SPEG-less hearts come under further scrutiny.

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References

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