The Novel Roles of Four and A Half LIM Proteins 1 and 2 in the Cardiovascular System

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Four and a half LIM domains protein 1 (FHL1) and FHL2, as the name suggests, contain four and a half LIM domain binding proteins. Proteins in this family capable of interacting with many types of proteins, including structural proteins, kinases, and several classes of transcription factors, have been identified. These interactions have been found to have important roles in a variety of fundamental processes including transcriptional regulation, cardiovascular development, hypertrophy, atherosclerosis, and angiogenesis. This article reviews recent advances in the characterization of FHL1 and FHL2, their biological roles, LIM domain binding proteins, and functions in the cardiovascular system. *(Chang Gung Med J 2011;34:127-34)*

Key words: cardiovascular system, FHL1, FHL2, LIM domain binding protein

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The cardiovascular system is a delicate and complex structure.[1,2] Cardiac muscle is equipped with intricate, intrinsic mechanisms to regulate adaptive remodeling, and vessels are responsible for transportation throughout the whole body. Many different types of proteins, including structural and signal networks, are present to maintain homeostasis and respond to environmental stresses. Most of the known proteins have been well studied, but there are many other novel proteins that play important roles in various situations to compensate the functions of the house-keeping proteins.[3] The LIM domain binding proteins,[4-6] whose functions remain unknown, may influence the cardiovascular system from embryonic developmental to end stage heart failure, depending on their expression patterns and alternative splicing forms.

LIM domain binding protein

The name of the LIM domain, is derived from three homeodomain proteins (Caenorhabditis elegans Lin-11, rat Isl-1, and C. elegans Mec-3). This domain has been recognized in a variety of cytoplasmic and nuclear functional molecules. It defines a double zinc finger structure containing a characteristic cysteine-rich sequence with about 50 amino acids, and has been known as a potent protein-protein interaction motif.[6-10] Through a variety of protein-protein interactions, the functions of LIM domain proteins in the nucleus are mainly in tissue-

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specific gene regulation and determination of cell fate, whereas cytoplasmic LIM domain proteins are mainly involved in cytoskeleton organization.[4] It is very interesting to dissect the basic mechanisms and phenotypes of the LIM domain binding proteins in murine models.

Murine model of cardiovascular LIM domain binding protein

An *MLP*-null mouse (lack of muscle-enriched LIM domain proteins) demonstrated severe cardiac dysfunction and histological changes closely resembling those in human dilated cardiomyopathy interactions with the Z disk/titin network.[11,12] Another mouse model deficient in actinin-associated LIM domain protein was established which revealed a developmental pathway for right ventricular cardiomyopathy.[13]

Many LIM domain binding proteins have been studied with gene-engineered murine models to elucidate the functions of LIM proteins in our laboratory.[3,8-10,14-26] A search of the mouse expressed sequence tags (EST) database with the LIM domain of MLP revealed multiple EST clones corresponding to the cDNAs encoding four-and-half LIM 1 (FHL1), FHL2,[7,9] and other LIM domain proteins containing cDNAs. Newly identified mechanical stress sensors are not limited to the Z-disk region and to the I-band and M-band regions of titin, but are also embedded in muscle-specific membrane systems such as the costamere, intercalated disks, and caveolae-like microdomains.[6] This review summarizes current knowledge of FHL1 and FHL2 with a focus on how the cardiovascular system adjusts its pathophysiological remodeling process to meet mechanical demands.

Cardiovascular pathophysiology

Developmental cardiology

In the early development of the heart (embryonic day [E] 8.5), FHL1 expression has been observed strongly in the developing outflow tract, and to a lesser extent in the myocardium.[9] FHL2, E8.0, appears to have the most restricted expression pattern during development, in the heart, blood vessels, and skeletal muscle., FHL2 locates in vascular smooth muscle and endothelial cells in vessels as shown by Northern blot and knock-in LacZ cDNA for potential lineage tracking.[7,8] Expression of FHL1 in the heart is highest in the cardiac septa and in the region adjacent to the atrioventricular ring, suggesting a potential role in septation or conduction system development.[9] In addition, abrogation of FHL1 expression in primary human pulmonary artery smooth muscle cells has been reported.[27]

FHL1 has been recognized as a novel regulator of myosin-binding protein C (MyBP-C) activity, and plays a role in sarcomere assembly.[28] FHL1 and MyBP-C not only form a complex, but FHL1 may extend into the C-zone of the A-band, or inhibit the association of MyBP-C.[28] Overexpression of FHL1 in differentiating skeletal myoblasts induces the formation of multinucleated myosacs, which is associated with disruption of the Z-line and myosin thick filaments.[28] FHL1 localizes to the I-band/Z-line region and to the M-line in both mature skeletal muscle and isolated myofibrils. Moreover, FHL1 localizes to focal adhesions in skeletal myoblasts, where it promotes integrin-dependent cell spreading and migration.[29] FHL2 also localizes to the I-band and faintly to the M-line in isolated cardiomyocytes where it binds titin and scaffolds several muscle metabolic enzymes.[28,29] There is one immunohistochemistry study that has shown that FHL2 colocalizes with α-actinin at the Z-lines of human cardiac myocytes.[30]

Cardiac hypertrophy

In striated muscle, LIM proteins play critical roles in scaffolding, sarcomeric, and signaling proteins.[28] FHL1 expression is also increased in hypertrophied human hearts.[16,31-34] SLIMMER (FHL1B/KyoT3), a spliced isoform of FHL1, interacts with the proapoptotic protein Siva-1, and delays skeletal myoblast apoptosis.[35] FHL1 itself can increase muscle fiber size and oxidative slow fiber type expression, leading to increased muscle strength and endurance; these characteristics identify it as a regulator of skeletal muscle mass.[36] FHL1 transgenic mice exhibit skeletal muscle hypertrophy rather than hyperplasia, showing an approximately 20% increase in fiber size, but no change in fiber number, by enhancing nuclear fator of acrivated T-cells transcriptional activity.[36] Moreover, *FHL1* mRNA is increased in hypertrophy in both mice and humans.[9,34]

FHL2-null mice have been shown to have greater cardiac hypertrophic growth following catecholamine infusion.[37] However, when *FHL2*-null

mice were subjected to pressure overload stimulation by aortic banding, a hypertrophic response was observed that was indistinguishable from that of wild-type controls.[10] The discrepancy between these two studies is potentially because of the nature of the stimulus, in that pressure overload produces a more global response which recruits diverse hypertrophic signaling pathways, while catecholamine infusion may evoke a more unitary response that is dependent on MEK1-ERK1/2 signaling.[38]

Cardiac failure

FHL1 mRNA is increased in different mouse models of beta-adrenergic-induced cardiomyopathy.[31] In cardiac hypertrophy and dilated cardiomyopathy mouse models, cardiac ventricular expression of FHL-1, but not of related proteins FHL-2, or FHL-3, was altered.[9]

Reduction of FHL2 expression occurs in human left ventricular failure, and failure and knock-out cardiac titin containing a unique *N2B region* mice might lead to altered localization and reduced activity of metabolic enzymes.[39,40] Altered FHL2 expression in heart failure is associated with disruption of the normal subcellular localization of phosphofructokinase 2, adenylate kinase, and creatine kinase M isoform, and reduced activity of phosphofructokinase 2 and adenylate kinase, which might have important consequences for myocardial energy metabolism in heart failure.[39]

Angiogenesis

FHL2 expression has also been demonstrated in the lung and in vascular smooth muscle cells (vSMCs) of different origins, a finding that suggests a potential role of FHL2 in the vasculature.[5] FHL2 is a key regulator of vasomotor tone,[41] and *FHL2*-null mice exhibit significant elevations in systolic, diastolic, and mean blood pressure. Deletion of the FHL2 gene attenuated neovascularization after corneal injury, which indicates that the FHL2 protein plays a role in inhibiting inflammatory angiogenesis.[8] Similar findings were demonstrated in a mouse ischemic leg model with possible impaired stem cell mobility (our unpublished data).

Interestingly, *FHL1*-null mice exhibit aggravated angiogenesis with increased macrophage infiltration (our unpublished data). The mechanism requires further evaluation.

Atherosclerosis

Overexpression of FHL2 strongly inhibits vascular endothelial growth factor -induced endothelial cell migration and might regulate phosphatidylinositol 3-kinase/Akt via direct suppression of the sphingosine kinase-1- sphingosine-1-phosphate (SK1-S1P) pathway.[42] Recently, our work has demonstrated that FHL2 is expressed in the endothelium and smooth muscle of vessels, and in diet-induced hyperlipidemia.[7] Deletion of FHL2 is associated with resistance to atherogenesis and changes of endothelial cell function, including prevention of nitrogen oxidate suppression and vascular connexin downregulation. These results suggest that FHL2 is intimately associated with the regulation of endothelial cell function.[8]

FHL2 inhibits transcriptional activation of vSMC-specific genes mediated by the bone morphogenetic protein (BMP)-signaling pathway through the CArG box-binding proteins, such as serum response factor and members of the myocardin (Myocd) family.[41] This is a novel mechanism of regulation of SMC-specific contractile genes by FHL2. Aortic rings from homozygous *FHL2*-null mice display abnormalities in both endothelial-dependent and endothelial-independent relaxation, suggesting that FHL2 is essential for the regulation of vasomotor tone.

Although estrogen plays important roles in the pathophysiology of atherosclerosis and cardiovascular diseases mediated by estrogen receptor alpha (ERalpha), FHL2 mainly interacts with the N-terminal A/B domain of ERalpha, but not the C-terminal ligand-binding domain.[43] Overexpression of full-length FHL2 does not affect ERalpha-dependent transcriptional activities. The different functions of FHL2 may be observed in a cell type- or promoter-specific manner.[43]

Pulmonary hypertension

FHL1 has been observed in vascular smooth muscle cells, and is predominantly restricted to the cytoplasm.[9,27] Recently, FHL1 has been identified as a key protein in pulmonary hypertension through protein screening, and upregulation has been verified in idiopathic pulmonary arterial hypertension models.[27] Talin1 might act as an new interacting partner of FHL1.[27] Because FHL1 and FHL2 share high structural homology and some protein binding part-

ners, it is plausible that FHL2 as well as FHL1 might contribute to the pathogenesis of pulmonary hypertension by promoting vSMCs transition to a highly proliferative and migratory phenotype, or through modulation of BMP-mediated regulation of contractile genes.[27]

Arrhythmia

Using a fusion protein and mass spectrometry-based methods, the scaffolding protein FHL1 was identified as a potential protein partner for KCNA5.[44] Immunoprecipitation experiments, cotransfected cells, and confocal microscopy have demonstrated areas of colocalization after immunolabelling of both proteins. FHL1 also increased the extent and speed of K^+ current slow inactivation, with additional effects on the voltage dependence and recovery of this process. The role of FHL1 may be as a key molecular component in the I(Kur) complex in the human atrium, where it likely regulates functional expression of KCNA5.[44]

The highest levels of expression of both FHL1 and FHL2 during murine development are in the cardiac septa and in the region adjacent to the atrioventricular bundle,[9] a pattern which has also been noted for minK. Alternative splicing may affect the detailed expression patterns.[45] FHL2, a functionally important partner of the minK potassium channel subunit in heterologous cells, is required for the generation of I_K.[46] The identification of FHL2 as an I_K modifier makes it a candidate modulator for arrhythmia syndromes such as congenital long QT syndrome. Moreover, the interaction is specific for minK and does not extend to KvLQT1. The role of FHL2 is likely to link minK to the cytoskeleton, given that Z-lines are known to play a crucial role in the establishment and maintenance of cardiomyocyte cytoarchitecture.[46] The link between the I_Ks channel complex and the cytoskeleton is via the modular FHL2 protein.

Mechanism

Mice lacking FHL1 display a blunted hypertrophic response and a beneficial functional response to pressure overload induced by transverse aortic constriction, as described in our previous study.[10] A link to the Gαq (Gq) signaling pathway was also observed, as FHL1 deficiency prevented the cardiomyopathy observed in Gq transgenic mice.[16] Mechanistic studies have demonstrated that FHL1 plays an important role in the mechanism of pathological hypertrophy by sensing biomechanical stress responses via the N2B stretch sensor domain of titin and initiating changes in the titin- and mitogen- activated protein kinase- mediated responses important for sarcomere extensibility and intracellular signaling.[16]

FHL2 functions as an important transcriptional coactivator when present in the nucleus. For example, FHL2 has been shown to coactivate the androgen receptor activator protein-1 (AP-1), β-catenin, camp responsive element binding (CREB), camp responsive element modulator (CREM), and the transcriptional response downstream of wnt signaling.[47-58] FHL2 can serve a repressor function in cardiomyocytes through its ability to inhibit ERK1/2 transcriptional coupling.[38] Moreover, FHL2 is an SK1-interacting protein in both yeast and mammalian cells.[59] FHL2, but not FHL1 or FHL3, interacts with SK1 in the cytoplasm. The interaction sites with SK1 consist of at least 4 LIM domains in FHL2, whereas the C-terminal portion of SK1 mediates the binding of FHL2 in SK1. Overexpression of FHL2 attenuates the activity and anti-apoptotic effects of SK1.

Conclusion

Cytoskeletal proteins from the LIM domain family are thought to play a pivotal role in biomechanical stress responses leading to muscle disease. FHL1 and FHL2 are enriched in mammalian striated muscle, and associated with multiple binding partners and biological functions, including cardiovascular development and function.

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REFERENCES

1. Chien KR, Domian IJ, Parker KK. Cardiogenesis and the complex biology of regenerative cardiovascular medicine. Science 2008;322:1494-7.
2. Chien KR. Genomic circuits and the integrative biology of cardiac diseases. Nature 2000;407:227-32.
3. Chu PH, Lu JT, Chen J. Gene-engineered models for

genetic manipulation and functional analysis of the cardiovascular system in mice. Chang Gung Med J 2003;26: 868-78.
4. Zheng Q, Zhao Y. The diverse biofunctions of LIM domain proteins: determined by subcellular localization and protein-protein interaction. Biol Cell 2007;99:489-502.
5. Johannessen M, Moller S, Hansen T, Moens U, Van Ghelue M. The multifunctional roles of the four-and-a-half-LIM only protein FHL2. Cell Mol Life Sci 2006;63:268-84.
6. Hoshijima M. Mechanical stress-strain sensors embedded in cardiac cytoskeleton: Z disk, titin, and associated structures. Am J Physiol Heart Circ Physiol 2006;290:H1313-25.
7. Chu PH, Yeh HI, Wu HH, Hong RC, Shiu TF, Yang CM. Deletion of the FHL2 gene attenuates the formation of atherosclerotic lesions after a cholesterol-enriched diet. Life Sci 2010;86;365-71.
8. Chu PH, Yeh LK, Lin HC, Jung SM, Ma DH, Wang IJ, Wu HH, Shiu TF, Chen J. Deletion of the FHL2 gene attenuating neovascularization after corneal injury. Invest Ophthalmol Vis Sci 2008;49:5314-8.
9. Chu PH, Ruiz-Lozano P, Zhou Q, Cai C, Chen J. Expression patterns of FHL/SLIM family members suggest important functional roles in skeletal muscle and cardiovascular system. Mech Dev 2000;95:259-65.
10. Chu PH, Bardwell WM, Gu Y, Ross J Jr, Chen J. FHL2 (SLIM3) is not essential for cardiac development and function. Mol Cell Biol 2000;20:7460-2.
11. Arber S, Hunter JJ, Ross J Jr, Hongo M, Sansig G, Borg J, Perriard JC, Chien KR, Caroni P. MLP-deficient mice exhibit a disruption of cardiac cytoarchitectural organization, dilated cardiomyopathy, and heart failure. Cell 1997;88:393-403.
12. Knoll R, Hoshijima M, Hoffman HM, Person V, Lorenzen-Schmidt I, Bang ML, Hayashi T, Shiga N, Yasukawa H, Schaper W, McKenna W, Yokoyama M, Schork NJ, Omens JH, McCulloch AD, Kimura A, Gregorio CC, Poller W, Schaper J, Schultheiss HP, Chien KR. The cardiac mechanical stretch sensor machinery involves a Z disc complex that is defective in a subset of human dilated cardiomyopathy. Cell 2002;111:943-55.
13. Pashmforoush M, Pomies P, Peterson KL, Kubalak S, Ross J Jr, Hefti A, Aebi U, Beckerle MC, Chien KR. Adult mice deficient in actinin-associated LIM-domain protein reveal a developmental pathway for right ventricular cardiomyopathy. Nat Med 2001;7:591-7.
14. Cheng H, Kimura K, Peter AK, Cui L, Ouyang K, Shen T, Liu Y, Gu Y, Dalton ND, Evans SM, Knowlton KU, Peterson KL, Chen J. Loss of enigma homolog protein results in dilated cardiomyopathy. Circ Res 2010;107: 348-56.
15. Liang X, Sun Y, Ye M, Scimia MC, Cheng H, Martin J, Wang G, Rearden A, Wu C, Peterson KL, Powell HC, Evans SM, Chen J. Targeted ablation of PINCH1 and PINCH2 from murine myocardium results in dilated cardiomyopathy and early postnatal lethality. Circulation 2009;120:568-76.
16. Sheikh F, Raskin A, Chu PH, Lange S, Domenighetti AA, Zheng M, Liang X, Zhang T, Yajima T, Gu Y, Dalton ND, Mahata SK, Dorn GW, 2nd, Heller-Brown J, Peterson KL, Omens JH, McCulloch AD, Chen J. An FHL1-containing complex within the cardiomyocyte sarcomere mediates hypertrophic biomechanical stress responses in mice. J Clin Invest 2008;118:3870-80.
17. Sun Y, Liang X, Najafi N, Cass M, Lin L, Cai CL, Chen J, Evans SM. Islet 1 is expressed in distinct cardiovascular lineages, including pacemaker and coronary vascular cells. Dev Biol 2007;304:286-96.
18. Liang X, Sun Y, Schneider J, Ding JH, Cheng H, Ye M, Bhattacharya S, Rearden A, Evans S, Chen J. Pinch1 is required for normal development of cranial and cardiac neural crest-derived structures. Circ Res 2007;100:527-35.
19. Lai CF, Bai S, Uthgenannt BA, Halstead LR, McLoughlin P, Schafer BW, Chu PH, Chen J, Otey CA, Cao X, Cheng SL. Four and half lim protein 2 (FHL2) stimulates osteoblast differentiation. J Bone Miner Res 2006;21:17-28.
20. Govoni KE, Baylink DJ, Chen J, Mohan S. Disruption of four-and-a-half LIM 2 decreases bone mineral content and bone mineral density in femur and tibia bones of female mice. Calcif Tissue Int 2006;79:112-7.
21. Liang X, Zhou Q, Li X, Sun Y, Lu M, Dalton N, Ross J, Jr, Chen J. PINCH1 plays an essential role in early murine embryonic development but is dispensable in ventricular cardiomyocytes. Mol Cell Biol 2005;25:3056-62.
22. Huang C, Zhou Q, Liang P, Hollander MS, Sheikh F, Li X, Greaser M, Shelton GD, Evans S, Chen J. Characterization and in vivo functional analysis of splice variants of cypher. J Biol Chem 2003;278:7360-5.
23. Cai CL, Liang X, Shi Y, Chu PH, Pfaff SL, Chen J, Evans S. Isl1 identifies a cardiac progenitor population that proliferates prior to differentiation and contributes a majority of cells to the heart. Dev Cell 2003;5:877-89.
24. Zhou Q, Chu PH, Huang C, Cheng CF, Martone ME, Knoll G, Shelton GD, Evans S, Chen J. Ablation of Cypher, a PDZ-LIM domain Z-line protein, causes a severe form of congenital myopathy. J Cell Biol 2001;155:605-12.
25. Zhou Q, Ruiz-Lozano P, Martone ME, Chen J. Cypher, a striated muscle-restricted PDZ and LIM domain-containing protein, binds to alpha-actinin-2 and protein kinase C. J Biol Chem 1999;274:19807-13.
26. Sheikh F, Bang ML, Lange S, Chen J. "Z"eroing in on the role of Cypher in striated muscle function, signaling, and human disease. Trends Cardiovasc Med 2007;17:258-62.
27. Kwapiszewska G, Wygrecka M, Marsh LM, Schmitt S, Trosser R, Wilhelm J, Helmus K, Eul B, Zakrzewicz A,

Ghofrani HA, Schermuly RT, Bohle RM, Grimminger F, Seeger W, Eickelberg O, Fink L, Weissmann N. Fhl-1, a new key protein in pulmonary hypertension. Circulation 2008;118:1183-94.
28. McGrath MJ, Cottle DL, Nguyen MA, Dyson JM, Coghill ID, Robinson PA, Holdsworth M, Cowling BS, Hardeman EC, Mitchell CA, Brown S. Four and a half LIM protein 1 binds myosin-binding protein C and regulates myosin filament formation and sarcomere assembly. J Biol Chem 2006;281:7666-83.
29. Lange S, Auerbach D, McLoughlin P, Perriard E, Schafer BW, Perriard JC, Ehler E. Subcellular targeting of metabolic enzymes to titin in heart muscle may be mediated by DRAL/FHL-2. J Cell Sci 2002;115:4925-36.
30. Li HY, Kotaka M, Kostin S, Lee SM, Kok LD, Chan KK, Tsui SK, Schaper J, Zimmermann R, Lee CY, Fung KP, Waye MM. Translocation of a human focal adhesion LIM-only protein, FHL2, during myofibrillogenesis and identification of LIM2 as the principal determinants of FHL2 focal adhesion localization. Cell Motil Cytoskeleton 2001;48:11-23.
31. Gaussin V, Tomlinson JE, Depre C, Engelhardt S, Antos CL, Takagi G, Hein L, Topper JN, Liggett SB, Olson EN, Lohse MJ, Vatner SF, Vatner DE. Common genomic response in different mouse models of beta-adrenergic-induced cardiomyopathy. Circulation 2003;108:2926-33.
32. Lim DS, Roberts R, Marian AJ. Expression profiling of cardiac genes in human hypertrophic cardiomyopathy: insight into the pathogenesis of phenotypes. J Am Coll Cardiol 2001;38:1175-80.
33. Hwang DM, Dempsey AA, Lee CY, Liew CC. Identification of differentially expressed genes in cardiac hypertrophy by analysis of expressed sequence tags. Genomics 2000;66:1-14.
34. Hwang DM, Dempsey AA, Wang RX, Rezvani M, Barrans JD, Dai KS, Wang HY, Ma H, Cukerman E, Liu YQ, Gu JR, Zhang JH, Tsui SK, Waye MM, Fung KP, Lee CY, Liew CC. A genome-based resource for molecular cardiovascular medicine: toward a compendium of cardiovascular genes. Circulation 1997;96:4146-203.
35. Cottle DL, McGrath MJ, Wilding BR, Cowling BS, Kane JM, D'Arcy CE, Holdsworth M, Hatzinisiriou I, Prescott M, Brown S, Mitchell CA. SLIMMER (FHL1B/KyoT3) interacts with the proapoptotic protein Siva-1 (CD27BP) and delays skeletal myoblast apoptosis. J Biol Chem 2009;284:26964-77.
36. Cowling BS, McGrath MJ, Nguyen MA, Cottle DL, Kee AJ, Brown S, Schessl J, Zou Y, Joya J, Bonnemann CG, Hardeman EC, Mitchell CA. Identification of FHL1 as a regulator of skeletal muscle mass: implications for human myopathy. J Cell Biol 2008;183:1033-48.
37. Kong Y, Shelton JM, Rothermel B, Li X, Richardson JA, Bassel-Duby R, Williams RS. Cardiac-specific LIM protein FHL2 modifies the hypertrophic response to beta-adrenergic stimulation. Circulation 2001;103:2731-8.
38. Purcell NH, Darwis D, Bueno OF, Muller JM, Schule R, Molkentin JD. Extracellular signal-regulated kinase 2 interacts with and is negatively regulated by the LIM-only protein FHL2 in cardiomyocytes. Mol Cell Biol 2004;24:1081-95.
39. Bovill E, Westaby S, Crisp A, Jacobs S, Shaw T. Reduction of four-and-a-half LIM-protein 2 expression occurs in human left ventricular failure and leads to altered localization and reduced activity of metabolic enzymes. J Thorac Cardiovasc Surg 2009;137:853-61.
40. Radke MH, Peng J, Wu Y, McNabb M, Nelson OL, Granzier H, Gotthardt M. Targeted deletion of titin N2B region leads to diastolic dysfunction and cardiac atrophy. Proc Natl Acad Sci USA 2007;104:3444-9.
41. Neuman NA, Ma S, Schnitzler GR, Zhu Y, Lagna G, Hata A. The four-and-a-half LIM domain protein 2 regulates vascular smooth muscle phenotype and vascular tone. J Biol Chem 2009;284:13202-12.
42. Hayashi H, Nakagami H, Takami Y, Koriyama H, Mori M, Tamai K, Sun J, Nagao K, Morishita R, Kaneda Y. FHL-2 suppresses VEGF-induced phosphatidylinositol 3-kinase/Akt activation via interaction with sphingosine kinase-1. Arterioscler Thromb Vasc Biol 2009;29:909-14.
43. Kobayashi S, Shibata H, Yokota K, Suda N, Murai A, Kurihara I, Saito I, Saruta T. FHL2, UBC9, and PIAS1 are novel estrogen receptor alpha-interacting proteins. Endocr Res 2004;30:617-21.
44. Yang Z, Browning CF, Hallaq H, Yermalitskaya L, Esker J, Hall MR, Link AJ, Ham AJ, McGrath MJ, Mitchell CA, Murray KT. Four and a half LIM protein 1: a partner for KCNA5 in human atrium. Cardiovasc Res 2008;78:449-57.
45. Ng EK, Lee SM, Li HY, Ngai SM, Tsui SK, Waye MM, Lee CY, Fung KP. Characterization of tissue-specific LIM domain protein (FHL1C) which is an alternatively spliced isoform of a human LIM-only protein (FHL1). J Cell Biochem 2001;82:1-10.
46. Kupershmidt S, Yang IC, Sutherland M, Wells KS, Yang T, Yang P, Balser JR, Roden DM. Cardiac-enriched LIM domain protein fhl2 is required to generate I(Ks) in a heterologous system. Cardiovasc Res 2002;56:93-103.
47. Du X, Hublitz P, Gunther T, Wilhelm D, Englert C, Schule R. The LIM-only coactivator FHL2 modulates WT1 transcriptional activity during gonadal differentiation. Biochim Biophys Acta 2002;1577:93-101.
48. Fimia GM, De Cesare D, Sassone-Corsi P. A family of LIM-only transcriptional coactivators: tissue-specific expression and selective activation of CREB and CREM. Mol Cell Biol 2000;20:8613-22.
49. Kollara A, Brown TJ. Modulation of aryl hydrocarbon receptor activity by four and a half LIM domain 2. Int J Biochem Cell Biol 2009;41:1182-8.
50. Muller JM, Metzger E, Greschik H, Bosserhoff AK, Mercep L, Buettner R, Schule R. The transcriptional coactivator FHL2 transmits Rho signals from the cell

membrane into the nucleus. EMBO J 2002;21:736-48.
51. McLoughlin P, Ehler E, Carlile G, Licht JD, Schafer BW. The LIM-only protein DRAL/FHL2 interacts with and is a corepressor for the promyelocytic leukemia zinc finger protein. J Biol Chem 2002;277:37045-53.
52. Shi X, Bowlin KM, Garry DJ. Fhl2 interacts with Foxk1 and corepresses Foxo4 activity in myogenic progenitors. Stem Cells 2010;28:462-9.
53. Hamidouche Z, Hay E, Vaudin P, Charbord P, Schule R, Marie PJ, Fromigue O. FHL2 mediates dexamethasone-induced mesenchymal cell differentiation into osteoblasts by activating Wnt/beta-catenin signaling-dependent Runx2 expression. FASEB J 2008;22:3813-22.
54. Martin B, Schneider R, Janetzky S, Waibler Z, Pandur P, Kuhl M, Behrens J, von der Mark K, Starzinski-Powitz A, Wixler V. The LIM-only protein FHL2 interacts with beta-catenin and promotes differentiation of mouse myoblasts. J Cell Biol 2002;159:113-22.
55. Morlon A, Sassone-Corsi P. The LIM-only protein FHL2 is a serum-inducible transcriptional coactivator of AP-1. Proc Natl Acad Sci USA 2003;100:3977-82.
56. Muller JM, Isele U, Metzger E, Rempel A, Moser M, Pscherer A, Breyer T, Holubarsch C, Buettner R, Schule R. FHL2, a novel tissue-specific coactivator of the androgen receptor. EMBO J 2000;19:359-69.
57. Labalette C, Renard CA, Neuveut C, Buendia MA, Wei Y. Interaction and functional cooperation between the LIM protein FHL2, CBP/p300, and beta-catenin. Mol Cell Biol 2004;24:10689-702.
58. Wei Y, Renard CA, Labalette C, Wu Y, Levy L, Neuveut C, Prieur X, Flajolet M, Prigent S, Buendia MA. Identification of the LIM protein FHL2 as a coactivator of beta-catenin. J Biol Chem 2003;278:5188-94.
59. Sun J, Yan G, Ren A, You B, Liao JK. FHL2/SLIM3 decreases cardiomyocyte survival by inhibitory interaction with sphingosine kinase-1. Circ Res 2006;99:468-76.

全新蛋白 FHL1 及 FHL2 於心血管系統之地位

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Four-and-half LIM 1 (FHL1) 和 FHL2 包含四個半 LIM 結構蛋白，這一全新蛋白能與許多不同類型的蛋白質，包括結構蛋白激酶和其他類之轉錄蛋白進行交互的作用。而 FHL1 及 FHL2 這些交互作用已經發現的包括轉錄調控、心血管發育學、心肌肥厚、動脈粥狀硬化和血管新生成的各種重要的角色。這篇綜論特別在心血管系統中檢視 FHL1 和 FHL2 蛋白質之生物作用，和其特有之功能的新研究與新進展。(長庚醫誌 2011;34:127-34)

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