DOI:10.3724/SP.J.1008.2012.00540

·研究快报·

大鼠非梗死区心肌 Dock1 蛋白表达改变

刘小兰,赵文菊,李 刚*,王志华,王丽平
重庆医科大学附属第一医院老年科心血管病组,重庆 400016

[摘要] **目的** 探讨大鼠非梗死区心肌 Dock1 蛋白的表达改变。**方法** 建立 SD 大鼠实验性心肌梗死(22 只)及假手术模型(20 只)。分别于术后 24 h 和 12 周时处死心肌梗死组和假手术组大鼠(各 8 只),获取心脏标本,应用免疫印迹法检测非梗死区心肌及假手术组心肌 Dock1 蛋白的表达。**结果** 术后 24 h 时,心肌 Dock1 蛋白表达在梗死组(0.13±0.03)与假手术组(0.10±0.04)间的差异无统计学意义($P>0.05$);但术后 12 周时,梗死组(0.17±0.04)较假手术组(0.11±0.05)表达增加,差异有统计学意义($P<0.05$);术后 12 周时的梗死组心肌 Dock1 蛋白表达较 24 h 时的梗死组和假手术组均增加,差异有统计学意义($P<0.05$)。**结论** 心肌中存在 Dock1 蛋白的表达;梗死后 12 周时非梗死区心肌 Dock1 蛋白表达增加。

[关键词] Dock1 蛋白;鸟苷交换因子;心肌梗死;心肌
[中图分类号] R 542.22 [文献标志码] A [文章编号] 0258-879X(2012)05-0540-04

Change of Dock1 protein expression in non-infarcted myocardium in rats

LIU Xiao-lan, ZHAO Wen-ju, LI Gang*, WANG Zhi-hua, WANG Li-ping
Division of Cardiology, Department of Geriatrics, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China

[**Abstract**] **Objective** To investigate the change of Dock1 protein expression in non-infarcted myocardium in rats. **Methods** Experimental myocardial infarction models were established in 22 SD rats (MI group) and the other 20 rats received sham-operation (Sham group). Eight rats were sacrificed 24 h after operation in each group and another 8 were sacrificed at 12 weeks after operation; the cardiac specimens were collected. Dock1 protein expression was measured by Western blotting analysis in the non-infarcted myocardium of MI group and sham group. **Results** Dock1 protein expression was not significantly different in the non-infarcted myocardium between MI group (0.13±0.03) and Sham group (0.10±0.04) at 24 h after operation ($P>0.05$); 12 weeks after operation, the expression in non-infarcted myocardium of MI group (0.17±0.04) was significantly higher than that of Sham group (0.11±0.05, $P<0.05$). Dock1 protein expression in non-infarcted myocardium was significantly increased in MI group at 12 weeks after operation compared with those in MI and Sham groups at 24 h after operation ($P<0.05$). **Conclusion** There is Dock1 protein expression in rat's myocardium, and the expression is significantly increased in the non-infarcted myocardium at 12 weeks after infarction.

[**Key words**] Dock1 protein; guanine nucleotide exchange factor; myocardial infarction; myocardium
[Acad J Sec Mil Med Univ, 2012, 33(5): 540-543]

整合素(integrin)信号通路对心肌细胞的生长存活至关重要[1-2]。整合素 β_1 亚基和整合素连接激酶(integrin-linked kinase, ILK)可以降低大鼠非梗死区心肌细胞的凋亡,阻止梗死后心脏重塑、缺血性心肌病及心力衰竭的发生和发展[3-4]。鸟嘌呤核苷交换因子 Dock1(180 000 protein downstream of Crk)作为整合素通路的组分之一[1],推测其亦可影响心肌细胞的存活或凋亡,从而参与梗死后心脏重塑、缺血性心肌病及心力衰竭的发生和发展。本研究观察 Dock1 蛋白在非梗死区心肌中的表达,探讨其与心肌梗死(心梗)后心脏重塑、心肌纤维化、缺血性心肌病及心力衰竭的关系。

[收稿日期] 2012-02-13 [接受日期] 2012-03-19
[基金项目] 重庆市卫生局医学科学技术研究项目(2009-2-290, 04-2-154),重庆市科委自然科学基金(CSTC 2007BB5276). Supported by the Medical Science & Technology Research Project of Chongqing Health Bureau (2009-2-290, 04-2-154) and the Natural Science Foundation of Chongqing Science & Technology Commission (CSTC 2007BB5276).
[作者简介] 刘小兰,硕士,主治医师. E-mail: liuxiaolan99099@yahoo.com.cn
*通信作者(Corresponding author). Tel: 023-89012020, E-mail: ganglicqmu@126.com

1 材料和方法

1.1 材料 3～4 个月龄清洁级(SPF 级)雄性 SD 大鼠 50 只,购自重庆医科大学实验动物中心[SCXK(渝)2007-001],体质量(207.26±22.55) g;小型动物呼吸机购自上海精密仪器厂;彩色多普勒超声仪购自美国 GE 公司;SDS 裂解液、BCA 蛋白浓度测定试剂盒购自碧云天生物技术研究所;小鼠抗 β-actin 抗体(sc-47778)、小鼠 Dock1 抗体(sc-13163)购自 Santa Cruz 公司;辣根过氧化物酶标记的二抗购自北京中杉金桥生物技术有限公司。

1.2 模型制作与实验分组 随机选取大鼠,10%水合氯醛 3 ml/kg 腹腔注射麻醉,在左心耳下缘结扎冠状动脉左前降支,若心电图 ST 段抬高则提示心梗模型建立成功;假手术组在上述部位只穿线不结扎[5]。心梗组手术刚完成时仍存活的大鼠有 22 只,假手术组 20 只。术后均肌注青霉素 30 万单位,3 次/d,共 3 d,常规饲养。

1.3 心脏结构和功能 术后 24 h 和 12 周时,称质量,麻醉后用彩色多普勒超声仪测定左心室舒张末内径(LVIDd)、左心室射血分数(LVEF)、心率(HR)。然后分别随机处死心梗组大鼠 8 只,假手术组 8 只。取出心脏,沿房室沟去掉两心房和大血管后,沿室间沟剪下右心室,室间隔与左心室保留在一起,放入生理盐水中洗去血液,沾干,称质量(LV),得到左心室质量与体质量比(LV/BM,mg/g)。将左心室分成 2 份,含梗死区的心尖部放入 4%多聚甲醛固定,做组织学检测;靠心底的非梗死区心肌组织于−80℃保存,做蛋白质免疫印迹检测。

1.4 组织学检测 含梗死区的心尖部固定 24 h 后石蜡包埋,4 μm 切片,分别用苏木精-伊红(H-E)、苦味酸天狼星红染色,普通光镜下观察心肌组织结构,测胶原含量容积百分比(CVF)。随机选择非梗死区无血管区照相,用 Image-pro plus 6.0 软件进行 CVF 定量。

1.5 蛋白质免疫印迹检测 取非梗死区左心室心肌组织,提取蛋白,电泳,转膜,孵育一抗、二抗,显色。用 Quantity one 软件分析图像,以 Dock1 蛋白条带与 β 肌动蛋白条带光密度比值表示 Dock1 蛋白的相对表达水平。

1.6 统计学处理 计量数据以 $\bar{x}\pm s$ 表示,采用 SPSS 19.0 统计软件包进行统计分析,组间比较采用方差分析,两两比较采用 LSD 法。检验水平(α)为 0.05。

2 结 果

2.1 心脏结构和心功能改变 术后 24 h 梗死组较假手术组 LV/BM 增加($P<0.05$)。术后 12 周时梗死组较假手术组 LVIDd 增大,LV/BM 增加,LVEF 降低($P<0.05$)。术后 12 周时梗死组和假手术组与 24 h 比较,LVIDd 均增大,LV/BM 均降低;而 LVEF 仅梗死组降低($P<0.05$,表 1)。

表 1 各组大鼠心脏结构、功能和 CVF 改变

Tab 1 Changes of cardiac structure, function and collagen volume fraction (CVF) in rats of each group

$n=8$, $\bar{x}\pm s$

Group	24 h after ligation of LAD				
	LVIDd d/mm	LV/BM m_B/(mg·g^{-1})	LVEF	HR f/min^{-1}	CVF
Sham	5.55±0.73	2.17±0.12	0.67±0.10	436±53	0.066±0.018
MI	5.67±0.66	2.45±0.14*	0.66±0.09	448±58	0.063±0.023
Group	12 weeks after operation				
	LVIDd d/mm	LV/BM m_B/(mg·g^{-1})	LVEF	HR f/min^{-1}	CVF
Sham	6.40±0.63*	1.72±0.16*	0.65±0.09	420±54	0.081±0.042
MI	7.46±0.48*△▲	1.94±0.15*△▲	0.44±0.09*△▲	425±52	0.162±0.058*△▲

Sham: Sham operation group; MI: Myocardial infarction group; LAD: Left anterior descending branch of coronary artery; LVIDd: Left ventricular internal diameter at end-diastole; LV/BM: Left ventricular mass/body mass; LVEF: Left ventricular ejection fraction; HR: Heart rate. *$P<0.05$ vs sham group at 24 h after operation; △$P<0.05$ vs sham group at 12 weeks after operation; ▲$P<0.05$ vs MI group at 24 h after operation

2.2 组织学改变 术后 24 h 梗死组(图 1B)较假手术组(图 1A)心肌细胞明显肿胀,炎症细胞明显增加;术后 12 周梗死组(图 1D)较假手术组(图 1C)心肌细胞明显肥大。术后 24 h 假手术组与梗死组间 CVF 差异无统计学意义($P>0.05$);术后 12 周梗死组 CVF 高于假手术组($P<0.05$),且较 24 h 梗死组与假手术组增加($P<0.05$,表 1 和图 2)。

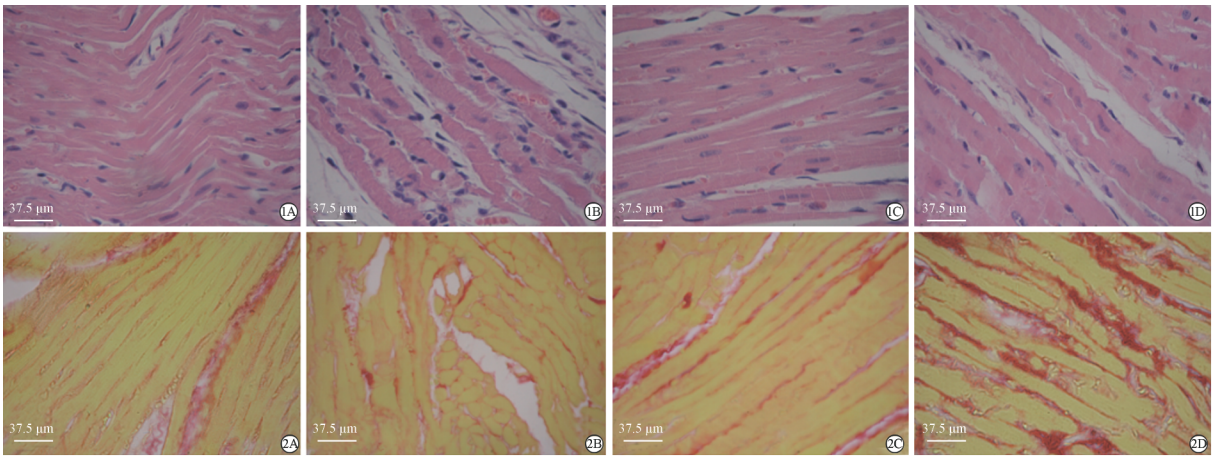

图 1　心肌 H-E 染色结果

Fig 1　Histology of myocardium by H-E staining

图 2　心肌苦味酸天狼星红染色结果

Fig 2　Histology of myocardium stained with picric acid & sirius red

A：Sham operation group at 24 h after operation；B：Myocardial infarction group at 24 h after operation；C：Sham operation group at 12 weeks after operation；D：Myocardial infarction group at 12 weeks after operation. Original magnification：×400

2.3　非梗死区心肌 Dock1 蛋白表达改变　术后 24 h，梗死组（0.13±0.03）与假手术组（0.10±0.04）心肌 Dock1 蛋白表达的差异无统计学意义（$P>0.05$）；但术后 12 周，梗死组心肌 Dock1 蛋白表达（0.17±0.04）较假手术组（0.11±0.05）增加（$P<0.05$），且较 24 h 时的梗死组和假手术组均增加（$P<0.05$，图 3）。

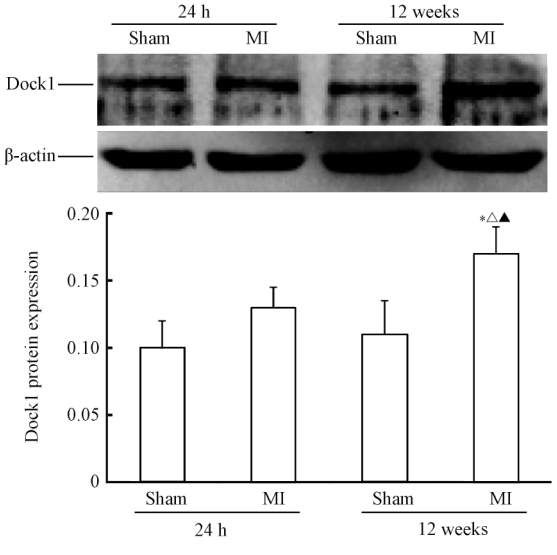

图 3　心肌 Dock1 蛋白表达改变

Fig 3　Change of Dock1 protein expression in rat myocardium

Sham：Sham operation group；MI：Myocardial infarction group；* $P<0.05$ vs Sham operation group at 24 h after operation；△ $P<0.05$ vs Sham operation group at 12 weeks after operation；▲ $P<0.05$ vs MI group at 24 h after operation. $n=8$，$\bar{x}\pm s$

3　讨　论

本研究主要发现：心肌中存在 Dock1 蛋白表达；且梗死后 12 周时，非梗死区心肌 Dock1 蛋白表达增加，心室腔明显扩大，心室质量增加，射血分数下降，心肌细胞肥大，胶原含量增加，心肌纤维化，提示存在心脏重塑及心力衰竭。

研究显示整合素是细胞膜上负责双向传递细胞信号的分子。它能把细胞外的机械张力信号转化为细胞内的生化信号，反之亦然[6]。研究显示心梗后非梗死区心肌整合素亚基表达增加[7]；而基因敲除整合素信号通路的整合素 β_1 亚基、ILK、FAK（focal adhesion kinase）等组分后可加速压力负荷诱导的心脏重塑及扩张型心肌病的发生、发展，使心肌细胞凋亡增加，生存力降低[1,3,8-10]。而过表达 ILK 可抑制心肌细胞凋亡，促进心肌细胞增殖、存活[11]，可减缓心梗后心脏的重塑，改善心脏功能[4]。提示整合素信号通路的整合素 β_1 亚基、ILK、FAK 等组分具有抑制心肌细胞凋亡，促进心肌细胞生存力的作用；可减缓心梗后心脏的重塑。

Dock1 亦是整合素信号通路的组分之一，研究发现它能与该信号通路的接头蛋白 Crk（CT10 regulator of kinase）的 SH3 结构域结合，形成 Dock1-Crk-p130Cas（Crk-associatedsubstrate）复合体，激

活 Rac GTP 酶,与细胞的伸展、迁移、细胞骨架调节等有关[12-14]。敲除 Dock1 可影响肌纤维的发育,导致骨骼肌量明显减少[15]和心血管发育严重障碍[16]。

本研究发现 Dock1 在成年大鼠心肌中存在表达。梗死后非梗死区心肌 Dock1 蛋白表达增加,该结果与文献报道相似[7],推测其与心梗后心脏重塑、心肌纤维化、缺血性心肌病和心力衰竭等引起的心脏腔室内张力增加及整合素信号通路激活有关。另外,亦提示 Dock1 参与了心梗后心脏重塑、心肌纤维化、缺血性心肌病和心力衰竭的发生、发展,但其机制仍然有待进一步阐明。

4 利益冲突

所有作者声明本文不涉及任何利益冲突。

[参 考 文 献]

[1] Lal H, Verma S K, Foster D M, Golden H B, Reneau J C, Watson L E, et al. Integrins and proximal signaling mechanisms in cardiovascular disease[J]. Front Biosci, 2009, 14: 2307-2334.

[2] Kuppuswamy D. Importance of integrin signaling in myocyte growth and survival[J]. Circ Res, 2002, 90: 1240-1242.

[3] Krishnamurthy P, Subramanian V, Singh M, Singh K. Deficiency of beta1 integrins results in increased myocardial dysfunction after myocardial infarction[J]. Heart, 2006, 92: 1309-1315.

[4] Ding L, Dong L, Chen X, Zhang L, Xu X, Ferro A, et al. Increased expression of integrin-linked kinase attenuates left ventricular remodeling and improves cardiac function after myocardial infarction[J]. Circulation, 2009, 120: 764-773.

[5] Mu Y, Li G, Wang Z H, Zhang C J. Up-regulation of phosphorylated ATM/ATR substrate/Akt expression by phenylephrine in peri-infarct myocardium in rats[J]. Acta Cardiol Sin, 2011, 27: 182-188.

[6] Srivastava D, Yu S. Stretching to meet needs: integrin-linked kinase and the cardiac pump[J]. Genes Dev, 2006, 20: 2327-2331.

[7] Nawata J, Ohno I, Isoyama S, Suzuki J, Miura S, Ikeda J, et al. Differential expression of alpha 1, alpha 3 and alpha 5 integrin subunits in acute and chronic stages of myocardial infarction in rats[J]. Cardiovasc Res, 1999, 43: 371-381.

[8] Umar S, van der Valk E J, Schalij M J, van der Wall E E, Atsma D E, van der Laarse A. Integrin stimulation-induced hypertrophy in neonatal rat cardiomyocytes is NO-dependent[J]. Mol Cell Biochem, 2009, 320(1-2): 75-84.

[9] Vadali K, Cai X, Schaller M D. Focal adhesion kinase: an essential kinase in the regulation of cardiovascular functions[J]. IUBMB Life, 2007, 59: 709-716.

[10] Lu H, Fedak P W, Dai X, Du C, Zhou Y Q, Henkelman M, et al. Integrin-linked kinase expression is elevated in human cardiac hypertrophy and induces hypertrophy in transgenic mice[J]. Circulation, 2006, 114: 2271-2279.

[11] Hannigan G E, Coles J G, Dedhar S. Integrin-linked kinase at the heart of cardiac contractility, repair, and disease[J]. Circ Res, 2007, 100: 1408-1414.

[12] Hasegawa H, Kiyokawa E, Tanaka S, Nagashima K, Gotoh N, Shibuya M, et al. DOCK180, a major CRK-binding protein, alters cell morphology upon translocation to the cell membrane[J]. Mol Cell Biol, 1996, 16: 1770-1776.

[13] Kiyokawa E, Hashimoto Y, Kobayashi S, Sugimura H, Kurata T, Matsuda M. Activation of Rac1 by a Crk SH3-binding protein, DOCK180[J]. Genes Dev, 1998, 12: 3331-3336.

[14] Kiyokawa E, Hashimoto Y, Kurata T, Sugimura H, Matsuda M. Evidence that DOCK180 up-regulates signals from the CrkⅡ-p130(Cas) complex[J]. J Biol Chem, 1998, 273: 24479-24484.

[15] Laurin M, Fradet N, Blangy A, Hall A, Vuori K, Côte J F. The atypical Rac activator Dock180 (Dock1) regulates myoblast fusion *in vivo*[J]. Proc Natl Acad Sci USA, 2008, 105: 15446-15451.

[16] Sanematsu F, Hirashima M, Laurin M, Takii R, Nishikimi A, Kitajima K, et al. DOCK180 is a Rac activator that regulates cardiovascular development by acting downstream of CXCR4[J]. Circ Res, 2010, 107: 1102-1105.

[本文编辑] 尹 荼