·基础研究·

脂联素对大鼠慢性间歇性低氧所致心肌重塑的影响

朱选风[1,2]，苏 梅[1]，丁文筱[1]，丁 宁[1]，黄 茂[1]，张希龙[1*]

（[1]南京医科大学第一附属医院呼吸科，南京 212029；[2]江苏省老年医院呼吸科，南京 212004）

【摘 要】**目的** 探讨慢性间歇性低氧（chronic intermittent hypoxia，CIH）对心肌重塑的影响及脂联素（adiponectin，Ad）的干预作用。**方法** 将45只Wistar大鼠随机分为3组：正常对照组（NC），CIH组和CIH+Ad组。CIH 35d后，使用马松染色分析方法检测左室纤维化程度及使用Western blot方法来衡量Ⅰ型胶原蛋白、Ⅲ型胶原蛋白和TGF-β/smad2/3通路蛋白的表达。通过RT-PCR方法来研究基质金属蛋白酶−2（MMP2）/基质金属蛋白酶的组织抑制剂−2（TIMP-2）的mRNA表达比值情况。**结果** 慢性间歇性低氧处理后，CIH组左心室的纤维化程度显著高于NC组和CIH + Ad组（$P < 0.05$），但NC组和CIH + Ad组之间差异具有统计学意义（$P < 0.05$）。CIH组Ⅰ型胶原蛋白和Ⅲ型胶原蛋白和MMP2/TIMP-2的mRNA比值表达最高，NC组表达最低，CIH + Ad组居中，3组之间均差异具有统计学意义（$P < 0.05$）。TGF-β/smad通路蛋白在CIH组中表达显著高于NC组和CIH组（$P < 0.05$），且NC组和CIH + Ad组差异具有统计学意义（$P < 0.05$）。**结论** 慢性间歇性低氧可引起左室重构，而Ad可能通过抑制TGF-β/smad2/3通路改善此损害。

【关键词】间歇性低氧；心功能紊乱；脂联素

【中图分类号】R341.5 【文献标识码】A 【DOI】10.3724/SP.J.1264.2014.000178

Effects of adiponectin on myocardiac remodeling induced by chronic intermittent hypoxia in Wistar rats

ZHU Xuan-Feng[1,2], SU Mei[1], DING Wen-Xiao[1], DING Ning[1], HUANG Mao[1], ZHANG Xi-Long[1*]

([1]Department of Respiratory Diseases, the First Affiliated Hospital, Nanjing Medical University, Nanjing 210029, China; [2]Department of Respiratory Diseases, Jiangsu Provincial Geriatrics Hospital, Nanjing 212004, China)

【**Abstract**】**Objective** To investigate the effects of chronic intermittent hypoxia (CIH) on myocardiac remodeling and intervention role of adiponectin (Ad) in the process. **Methods** A total of 45 Wistar rats were randomly divided into 3 groups: normal control (NC) group, CIH group and CIH + Ad group (10 μg Ad injection through caudal vein twice per week, for 5 weeks). After 35 days' CIH exposure (in a chamber with 60 s nitrogen infusion to reduce the oxygen concentration to 5% followed by another 60 s oxygen infusion to increase the concentration to 20% for totally 8 h of each day), Masson analysis was used to detect the left ventricular fibrosis, and Western blot was used to measure the protein expression of collagen Ⅰ, collagen Ⅲ and pathway proteins TGF-β/smad2/3. RT-PCR was used to study the expression of matrix metalloproteinase-2 (MMP-2) and tissue inhibitor of metalloproteinase-2 (TIMP-2). **Results** After CIH exposure, the left ventricular fibrosis was significantly more severe in CIH group than in NC group and CIH + Ad group ($P < 0.05$), and there was statistical difference between the 2 latter groups ($P < 0.05$). In addition, the protein expression of collagen Ⅰ and collagen Ⅲ and the mRNA expression of MMP-2 and TIMP-2 were the highest in CIH group, the lowest in NC group, and CIH + Ad group in the middle. There was a significant difference among 3 groups (all $P < 0.05$). The pathway proteins TGF-β/smad2/3 were most significantly expressed in CIH group ($P < 0.05$) , but there was still significant difference in their expression between NC group and CIH + Ad group ($P < 0.05$). **Conclusion** CIH may cause may left ventricular remodeling, while Ad supplement may ameliorate the impairment *via* inhibiting TGF-β/smad2/3 pathway.

【**Key words**】 intermittent hypoxia, cardiac dysfunction, adiponectin

This work was supported by the General Program of National Natural Science Foundation of China (81273571, 81370184).

Corresponding author: ZHANG Xi-Long, E-mail: zhangxilong1952@163.com

阻塞性睡眠呼吸暂停低通气综合征（obstructive sleep apnea hypopnea syndrome，OSAHS）作为一种现代常见病，其病理特征为睡眠期反复发生上气道塌陷，导致体内的慢性间歇性低氧（chronic intermittent

收稿日期：2014−06−22；修回日期：2014−07−10

基金项目：国家自然科学基金面上项目（81273571, 81370184）

通信作者：张希龙，E-mail: zhangxilong1952@163.com

hypoxia，CIH）进而可引起多种OSAHS并发症，其中以心血管并发症最为常见，已成为中老年心血管疾病的常见诱因之一。已有报道OSAHS与心功能紊乱有关而持续气道正压（continuous positive airway pressure，CPAP）治疗OSAHS可改善该心功能紊乱[1−3]。然而，OSAHS与心功能紊乱的相关机制尚不完全明了，有待探讨和发现有效的治疗方法。由于诸多OSAHS患者尚不能依从CPAP治疗，Matsumoto等[4]报道间歇性低氧可诱导左心室重构。其他报道也显示氧化应激、TGF-β和炎症因子可能在左心室重构中起到重要的作用[5]。Kim等[6]报道阻塞性睡眠呼吸左心室结构和功能重构有关。

脂联素（adiponectin，Ad）是新近发现的由脂肪细胞特异性分泌的一种蛋白质，是所有脂肪细胞因子中唯一负性调节的激素，具有降糖、增加胰岛素敏感性、抗炎、抗动脉硬化等作用[7−9]，与冠心病等疾病密切相关，已成为近年来医学领域的研究热点之一[10]。新近研究发现Ad与OSAHS关系密切，OSAHS患者存在低Ad血症，且CIH可导致低Ad血症，提示Ad极可能在OSAHS发病、发展中起着不可忽视的作用[11]。

已有报道Ad具有心脏保护作用，Ad通过激活PPARα可以防止血管紧张素Ⅱ诱导的心肌纤维化[12]。然而，CIH与左心室重构之间的关系以及Ad的可能干预作用尚未阐明。本研究通过建立大鼠的CIH模型探讨相关作用和机制。

1 材料与方法

1.1 实验动物

选取的45只成年雄性Wistar大鼠（SPF级）由南京医科大学实验动物中心提供。大鼠饲养在恒温（24℃）鼠房，12h：12h昼夜循环。大鼠可自由接触食物和水。这项研究经过南京医科大学动物伦理委员会的许可。

1.2 CIH造模

45只大鼠随机分为3组：对照组（normal control group，NC）、CIH组及CIH + Ad组。将CIH和CIH + Ad组大鼠饲养在一个特殊的玻璃舱内，连接一个气体控制系统，控制氮气和空气进入舱内。120s为一个循环，前60s冲入氮气，使舱内氧气浓度下降到5%，后面60s冲入空气，使氧气浓度逐渐恢复到20%。8h/d，持续35d。NC组大鼠放入相同的玻璃舱，持续冲入空气。同时，CIH + Ad组大鼠接受Ad治疗，2次/周，持续5周。每只大鼠每次尾静脉注射Ad 10μg，NC和CIH组大鼠尾静脉注射生理盐水。

1.3 组织处理

造模结束，使用戊巴比妥麻醉大鼠。打开胸腔，将心脏组织迅速分离，一部分被保存在−70℃，另一部分保存在4%多聚甲醛中。

1.3.1 Masson染色分析 使用Masson染色分析来检测心脏纤维化程度。将心脏组织脱水，包埋后，使用切片机切成5μm厚的石蜡切片。切片脱蜡和水化后，将其在R1中孵育1min，然后在R2中孵育30s，接下来在R3中孵育8min。最后在R4中孵育5min，封片，镜检。为了评估心脏组织的纤维化程度，每张切片取10个高倍镜视野（400×）。

1.3.2 实时定量RT-PCR分析 将100mg左心室组织加入1ml TRIzol试剂（Invitrogen，USA）后匀浆，按照TRIzol法提取总的RNA。大鼠基质金属蛋白酶（matrix metalloproteinase，MMP）−2引物序列（Invitrogen，USA）：正向（5'-AGGGCACCTCTTACAACAGC-3'）；反向（5'-CCCGGTCATAATCCTCGGTG-3'）。大鼠TIMP-2（Invitrogen，USA）引物序列：正向（5'-CAACCCCATCAAGAGGATTC-3'）；反向（5'-CGCAAGAACCATCACTTCTC-3'）。大鼠β-actin（Invitrogen，USA）引物序列：正向（5'-CAGGGTGTGA TGGTGGGTATGG-3'）；反向（5'-AGTTGGTGACAATGCCGTGTTC-3'）。所有基因PCR扩增的荧光信号都以β-actin作为对照，使用$2^{-\triangle\triangle CT}$法进行统计分析。

1.3.3 Western blot分析 使用含有氧化硫酰苯甲醇（PMSF）（1mmol/L）和磷酸酶抑制剂混合物（Roche，Germany）的组织蛋白提取试剂（Thermo Scientific，USA）提取左心室蛋白。将30mg组织放入300μl裂解液中制成匀浆。然后将匀浆物离心（10 000×g）5min，收集上清液即为左心室蛋白。10%凝胶分离总蛋白，各组上样量均为30μg。将凝胶上的蛋白电转移到PVDF膜上。用含5%BSA、0.1%Tween-20，pH为7.6的TBS室温封闭1h。然后将PVDF膜置于β-actin、胶原蛋白（collagen）Ⅰ、Ⅲ、TGF-β、smad2/3兔单抗缓冲液于4℃过夜。再用辣根过氧化物酶标记的羊抗兔孵育2h。最后使用增强型ECL试剂盒（Thermo Scientific，USA）检测条带，并利用数字成像系统（Image lab）进行灰度值分析。

1.4 统计学处理

应用SPSS统计软件进行分析。实验数据以$\bar{x} \pm s$表示。NC、CIH、CIH+Ad三组间心肌纤维化

的表达、Western印迹（灰度值）、mRNA表达水平的比较（计量资料）采用单因素方差分析法，组间两两比较采用q检验。$P<0.05$表示差异有统计学意义。

2 结 果

2.1 CIH对心肌纤维化的影响

CIH第35天结束时，心肌组织经Masson染色分析显示，CIH组的左心室心肌纤维化面积较NC组和CIH + Ad组显著扩大（$P<0.05$），NC组和CIH + Ad组之间差异亦具有统计学意义（$P<0.05$；图1）。以Western blot方法检测的左心室心肌胶原蛋白Ⅰ和胶原蛋白Ⅲ的表达在CIH组最高，NC组最低而CIH + Ad组居中。3组间差异均有统计学意义（$P<0.05$；图2）。

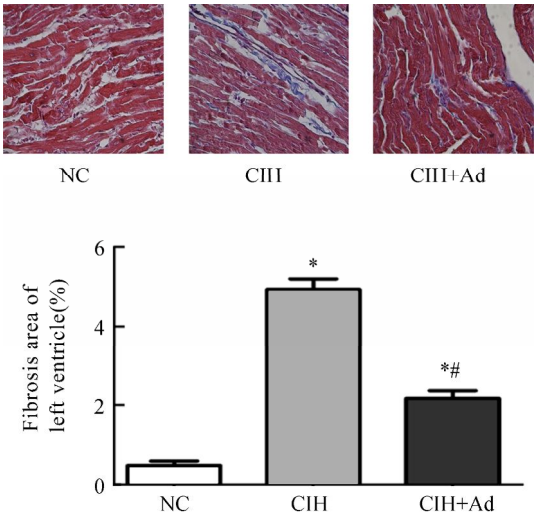
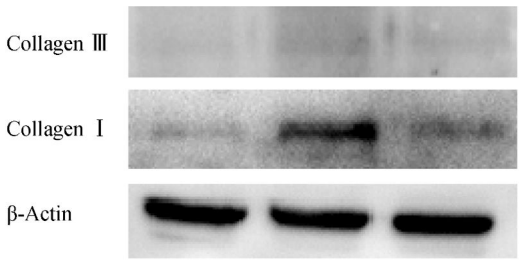


图1 左心室心肌Masson染色分析

Figure 1 The Masson analysis of the left ventricular myocardium(400 ×)
The blue represents the fibrosis and the red represents the normal myocardium. NC: normal control group; CIH: chronic intermittent hypoxia group; CIH+Ad: chronic intermittent hypoxia and adiponectin group. Compred with NC group, $^{*}P<0.05$; compared with CIH group, $^{\#}P<0.05$

2.2 与CIH诱导心肌纤维化的相关分子表达

在发现CIH可诱导左心室心肌纤维化后，我们进一步探讨了TGF-β/smad2/3通道是否与此纤维化有关。以Western blot方法检测TGF-β和磷酸化smad2/3发现，尽管NC和CIH + Ad组之间差异具有统计学意义（$P<0.05$），但CIH组较其他两组均显著增高（$P<0.05$；图3）。我们还检测到基质金属蛋白酶−2（matrix metalloproteinase 2，MMP-2）和MMP-9的基因水平虽然NC和CIH + Ad组之间差异具有统计学意义（$P<0.05$），但CIH组较其他两组均显著增高（均$P<0.05$）。此外，基质金属蛋白酶的组织抑制剂−1（tissue inhibitor of metalloproteinases 1，



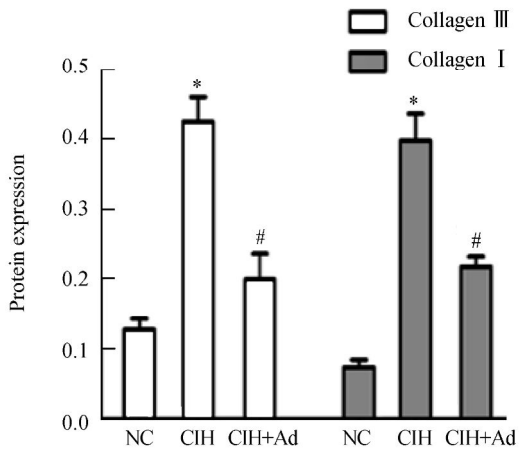


图2 胶原蛋白Ⅰ和胶胶原蛋白Ⅲ的蛋白质水平

Figure 2 The protein levels of collagen Ⅰ and collagen Ⅲ
NC: normal control group; CIH: chronic intermittent hypoxia group; CIH+Ad: chronic intermittent hypoxia and adiponectin group. Western blot bands of collagen Ⅰ and collagen Ⅲ were normalized to β-actin. Compared with NC group, $^{*}P<0.05$; compared with CIH group, $^{\#}P<0.05$

TIMP-1）和TIMP-2的基因表达水平在NC组最高，CIH组最低，而CIH + Ad组居中。3组间差异具有统计学意义（$P<0.05$；图4）。

3 讨 论

本研究通过建立大鼠CIH模型探讨了Ad对CIH左心室重构的影响及Ad的干预作用，结果发现在35d的CIH暴露之后，CIH的确可以诱导出左心室重构，表现为心肌纤维化的面积增大以及心肌组织中胶原蛋白Ⅰ和Ⅲ的蛋白表达增强。而在同样暴露于CIH但补充了外源性Ad的CIH+Ad组，左心室重构现象得到缓解。其机制可能与Ad抑制了TGF-β/smad2/3通路有关。

众所周知OSAHS是多种心血管疾病的危险因素[12]。已有报道提示CIH可以诱导小鼠或大鼠发生左心室重构[4,13−15]。本研究证实了该病理改变，发现了CIH组大鼠增强的左心室纤维化。由于我们曾发现CIH可诱导氧化应激和心肌细胞凋亡[16]，因而推测氧化应激可能也是形成左心室重构的重要因素[17]。然而，随着对暴露于CIH的大鼠补充外源性Ad，本研究又发现心肌纤维化的指标得到改善。Ad具备心肌保护作用已有较多报道[10,18−20]。Fujita等[12]

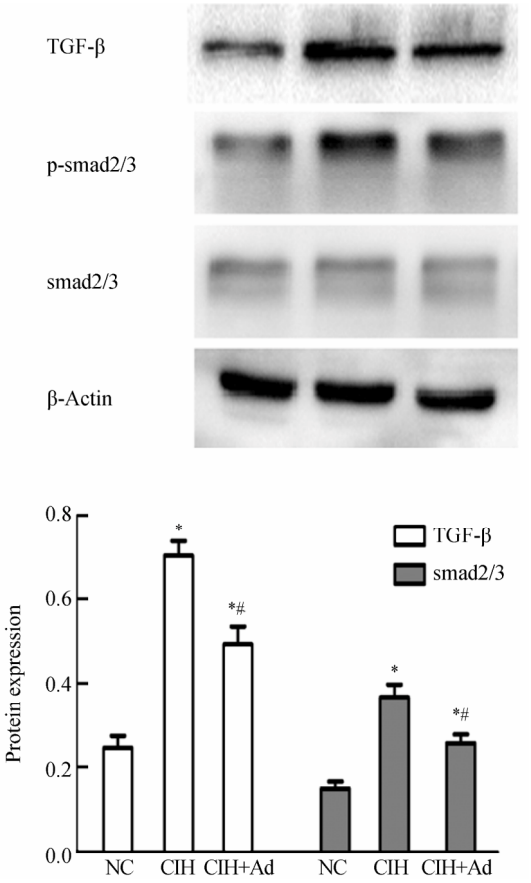


图3 TGF-β/smad2/3通道的蛋白水平

Figure 3 The protein levels of TGF-β/smad2/3 pathway

Western blot bands of TGF-β and smad2/3 were normalized to β-actin. Compared with NC group, $^{*}P < 0.05$; Compared with CIH group, $^{\#}P < 0.05$

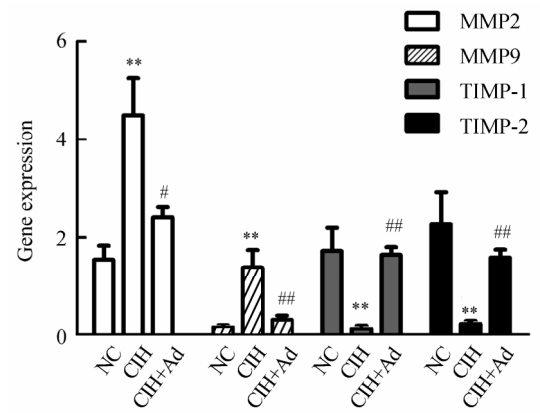


图4 3组间心肌组织的MMP-2，MMP-9，TIMP-1和TIMP-2的mRNA表达水平

Figure 4 The mRNA levels of MMP-2, MMP-9, TIMP-1 and TIMP-2 in myocardial tissues of three groups

PCR fluorescence signals of MMP-2, MMP-9, TIMP-1and TIMP-2 were normalized those of β-actin. NC: normal control group; CIH: chronic intermittent hypoxia group; CIH+Ad: chronic intermittent hypoxia and adiponectin group; TIMP-1: tissue inhibitor of metalloproteinases 1; TIMP-2:tissue inhibitor of metalloproteinases 2; MMP-2: matrix metalloproteinase 2; MMP-9: matrix metalloproteinase 9. Compared with NC group, $^{**}P < 0.01$; compared with CIH group, $^{\#}P < 0.05$; compared with CIH group, $^{\#\#}P < 0.01$

研究发现，Ad能够保护心肌，防止由血管紧张素Ⅱ诱导的心肌纤维化发生。Shimano等[21]的研究显示，Ad基因敲除的小鼠与WT小鼠相比，在TAC手术后表现出明显增强的左心室间充质纤维化。因而提示Ad可能缓解心肌纤维化和心脏质量（heart mass，HS）/体质量（body mass，BS）比率。

MMPs作为降解心肌细胞外蛋白的一族蛋白溶解酶，是一类与左心室重构相关的蛋白质，从mRNA水平可以检测出MMPs是否增高[4,22]。与以往的报道相似，本研究发现CIH可以诱导MMP-2和MMP-9的mRNA水平增高。TIMP系一种局部合成的蛋白质，附着于活化的MMPs来调控蛋白水解活性[22]。在暴露于CIH35d后，TIMP-1和TIMP-2的mRNA水平降低了，而在补充了Ad的大鼠中，尽管同样暴露于CIH，TIMP-1和TIMP-2的mRNA水平降低却部分改善了，该现象提示Ad保护心肌防止纤维化。

本研究结果显示，Ad能改善CIH诱导的左心室重构。TGF-β，作为一种多效细胞因子，与许多生物过程如细胞生长和分化、胚胎发育、纤维化、细胞增殖与生存和调节炎症反应等有关[23]。TFG-β信号经过跨膜受体激活smad2/3磷酸化，使磷酸化的smad2/3复合体转位于细胞核内，然后诱导纤维前靶基因表达[23-25]。有报道显示[24]，TGF-β信号在心脏重构中起到重要的作用，而且TGF-β的过表达能增强细胞间基质蛋白合成[26,27]。本研究发现CIH可以诱导TGF-β信号加强，而补充Ad却能减轻TGF-β与smad2/3的表达。

总之，本研究结果提示，Ad可以保护心肌，减轻CIH诱导的左心室重构。Ad的确切心脏保护机制还有待进一步研究予以阐明。

【参考文献】

[1] Kasai T. Sleep apnea and heart failure[J]. J Cardiol, 2012, 60(2): 78−85.

[2] Yasuma F, Ogihara A. Long-term treatment of ischemic dilated cardiomyopathy with continuous positive airway pressure[J]. Intern Med, 2001, 40(11): 1121−1127.

[3] Grewal RG. Treatment of cardiomyopathy with PAP therapy in a patient with severe obstructive sleep apnea[J]. J Clin Sleep Med, 2012, 8(5):581−583.

[4] Matsumoto C, Hayashi T, Kitada K, *et al*. Chymase plays an important role in left ventricular remodeling induced by intermittent hypoxia in mice[J]. Hypertension, 2009, 54(1): 164−171.

[5] Lijnen PJ, Petrov VV, Fagard RH. Induction of cardiac fibrosis by transforming growth factor-beta(1)[J]. Mol Genet Metab, 2000, 71(1−2): 418−435.

[6] Kim SM, Cho KI, Kwon JH, *et al*. Impact of obstructive sleep apnea on left atrial functional and structural remodeling beyond obesity[J]. J Cardiol, 2012, 60(6): 475−483.

[7] Arita Y, Kihara S, Ouchi N, *et al*. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity[J]. Biochem Biophys Res Commun, 1999, 257(1): 79−83.

[8] Pei H, Qu Y, Lu X, *et al*. Cardiac-derived adiponectin induced by long-term insulin treatment ameliorates myocardial ischemia/reperfusion injury in type 1 diabetic mice via AMPK signaling[J]. Basic Res Cardiol, 2013, 108(1): 322.

[9] Fruebis J, Tsao TS, Javorschi S, *et al*. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice[J]. Proc Natl Acad Sci USA, 2001, 98(4): 2005−2010.

[10] Ouchi N, Shibata R, Walsh K. Cardioprotection by adiponectin[J]. Trends Cardiovasc Med, 2006, 16(5): 141−146.

[11] Kosacka M, Korzeniewska A, Jankowska R. The evaluation of body composition, adiponectin, C-reactive protein and cholesterol levels in patients with obstructive sleep apnea syndrome[J]. Adv Clin Exp Med, 2013, 22(6): 817−824.

[12] Fujita K, Maeda N, Sonoda M, *et al*. Adiponectin protects against angiotensin Ⅱ-induced cardiac fibrosis through activation of PPAR-alpha[J]. Arterioscler Thromb Vasc Biol, 2008, 28(5): 863−870.

[13] Chen L, Einbinder E, Zhang Q, *et al*. Oxidative stress and left ventricular function with chronic intermittent hypoxia in rats[J]. Am J Respir Crit Care Med, 2005, 172(7): 915−920.

[14] Chen L, Zhang J, Gan TX, *et al*. Left ventricular dysfunction and associated cellular injury in rats exposed to chronic intermittent hypoxia[J]. J Appl Physiol (1985), 2008, 104(1): 218−223.

[15] Chen L, Zhang J, Hu X, *et al*. The Na^+/Ca^{2+} exchanger-1 mediates left ventricular dysfunction in mice with chronic intermittent hypoxia[J]. J Appl Physiol (1985), 2010, 109(6): 1675−1685.

[16] Ding W, Zhang X, Huang H, *et al*. Adiponectin protects rat myocardium against chronic intermittent hypoxia-induced injury *via* inhibition of endoplasmic reticulum stress[J]. PLoS One, 2014, 9(4): e94545.

[17] Inamoto S, Yoshioka T, Yamashita C, *et al*. Pitavastatin reduces oxidative stress and attenuates intermittent hypoxia-induced left ventricular remodeling in lean mice[J]. Hypertens Res, 2010, 33(6): 579−586.

[18] Amin RH, Mathews ST, Alli A, *et al*. Endogenously produced adiponectin protects cardiomyocytes from hypertrophy by a PPARgamma-dependent autocrine mechanism[J]. Am J Physiol Heart Circ Physiol, 2010, 299(3): H690−698.

[19] Shibata R, Sato K, Pimentel DR, *et al*. Adiponectin protects against myocardial ischemia-reperfusion injury through AMPK- and COX-2-dependent mechanisms[J]. Nat Med, 2005, 11(10): 1096−1103.

[20] Van Berendoncks AM, Garnier A, Ventura-Clapier R, *et al*. Adiponectin: key role and potential target to reverse energy wasting in chronic heart failure[J]. Heart Fail Rev, 2013, 18(5): 557−566.

[21] Shimano M, Ouchi N, Shibata R, *et al*. Adiponectin deficiency exacerbates cardiac dysfunction following pressure overload through disruption of an AMPK-dependent angiogenic response[J]. J Mol Cell Cardiol, 2010, 49(2): 210−220.

[22] Spinale FG. Matrix metalloproteinases: regulation and dysregulation in the failing heart[J]. Circ Res, 2002, 90(5): 520−530.

[23] Dobaczewski M, Chen W, Frangogiannis NG. Transforming growth factor (TGF)-β signaling in cardiac remodeling[J]. J Mol Cell Cardiol, 2011, 51(4): 600−606.

[24] Feng XH, Derynck R. Specificity and versatility in TGF-beta signaling through Smads[J]. Annu Rev Cell Dev Biol, 2005, 21: 659−693.

[25] Samarakoon R, Overstreet JM, Higgins PJ. TGF-β signaling in tissue fibrosis: redox controls, target genes and therapeutic opportunities[J]. Cell Signal, 2013, 25(1): 264−268.

[26] Eghbali M, Tomek R, Sukhatme VP, *et al*. Differential effects of transforming growth factor-beta 1 and phorbol myristate acetate on cardiac fibroblasts. Regulation of fibrillar collagen mRNAs and expression of early transcription factors[J]. Circ Res, 1991, 69(2): 483−490.

[27] Rosenkranz S, Flesch M, Amann K, *et al*. Alterations of beta-adrenergic signaling and cardiac hypertrophy in transgenic mice overexpressing TGF-beta(1)[J]. Am J Physiol Heart Circ Physiol, 2002, 283(3): H1253−H1262.

（编辑：李菁竹）