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Original Article

SIRT2 is down-regulated in myocardial infarction mice and regulates reparative angiogenesis via targeting AKT signaling pathway

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Abstract: Myocardial infarction (MI) remains one of the most deadly diseases worldwide with elusive mechanisms and limited treatment measures. Recent evidences showed that several histone deacetylase (HDAC) family proteins were involved in the pathogenesis of MI, especially in the process of reparative angiogenesis. Here, we first explored the expression level of SIRT2, an Nicotinamide Adenine Dinucleotide-Dependent deacetylase, in a mouse MI model. Then we further studied the function of SIRT2 on angiogenesis using human umbilical vein endothelial cells (HUVECs). As the result, we observed that the expression level of SIRT2 was down-regulated in endothelial cells isolated from ischemic but not remote myocardium early after MI. Knockdown of SIRT2 attenuated the angiogenesis ability of HUVECs, while overexpression of SIRT2 increased the angiogenesis ability. In addition, knockdown of SIRT2 decreased the level of phosphorylated AKT, which was a well-known modulator of reparative angiogenesis after MI in published literatures. Furthermore, the down-regulation of SIRT2 increased the acetylation level of AKT and β-catenin, suggesting the potential activity change in the both proteins. In conclusion, our results revealed that SIRT2 promoted reparative angiogenesis of HUVECs via targeting AKT signaling pathway. Reparative angiogenesis based on SIRT2 might provide theoretical basis for targeted therapy of MI patients.

Keywords: SIRT2, myocardial infarction, angiogenesis, AKT

Introduction

Myocardial infarction (MI) remains a global health issue and one of the deathliest diseases currently with limited therapeutic approaches [1, 2]. The progression of scar formation and left ventricular remodeling after MI are complex processes involving the interaction between fibroblasts and endothelial cells (ECs) where cardiomyocyte death often acts as a triggering event. The routine treatment measures for MI patients include procedural sedation and reperfusion therapy. With the wide application of percutaneous coronary intervention (PCI) in recent years, the symptoms of most MI patients could be effectively alleviated [3]. However, there still exists a large proportion of patients who are not suitable for PCI, especially those with diffuse or complete vascular thrombosis [4, 5]. Effective target interventions are long awaited to prevent recurrent cardiovascular events and complications to improve the dismal outcome of MI patients. Recently, a new therapeutic approach for MI patients has drawn much attention, namely the reparative angiogenesis. Angiogenesis-associated factors, including vascular endothelial growth factor (VEGF) family, angiopoietin family, and angiogenin family proteins have been identified and even tested in controlled clinical trials, while the upstream mechanisms determining angiogenesis remain elusive [6-8].

SIRT family protein contains a series of Nicotinamide Adenine Dinucleotide-dependent histone deacetylases [9]. As the most common category of sirtuin family, SIRT1 plays crucial roles in glucose synthesis, fatty acid oxidation as well as stress tolerance, indicating that SIRT1 might be involved in the pathogenesis of inflammation, diabetes and cardiovascular diseases [10-13]. As to MI, Potente and the colleges reported that SIRT1 could regulate endothelial-dependent angiogenesis [14]. Further-

Table 1. List of Oligonucleotides used in this study

Name	Sequeces (5'-3')	Note
SIRT2-Forward	5'-TCTGAGGTGACGCCCAAGTGT-3'	RT-PCR
SIRT2-Reverse	5'-TGCTGATGAGGGAGGCAAAGG-3'	RT-PCR
GAPDH-Forward	5'-AGCCACATCGCTCAGACAC-3'	RT-PCR
GAPDH-Reverse	5'-GCCCAATACGACCAAATCC-3'	RT-PCR
Non-targeting shRNA	5'-CCGGCAACAAGATGAAGAGCACCAACT CGAGTTGGTGCTCTTCATCTTGTTGTTTTTG-3'	shRNA cloning
SIRT2-sh1	5'-CCGGGCCATCTTTGAGATCAGCTATCTC GAGATAGCTGATCTCAAAGATGGCTTTTTGAATT-3'	shRNA cloning
SIRT2-sh2	5'-CCGGGCTAAGCTGGATGAAAGAGAACTC GAGTTCTCTTTCATCCAGCTTAGCTTTTTGAATT-3'	shRNA cloning

more, knockdown of SIRT1 exhibited obviously inhibitory effect on angiogenesis in *vitro* and ECs specific SIRT1 knockout mice showed angiogenic defect in ischemic tissues [15]. However, the biological role of SIRT2 in MI was still little known despite a recent study reported that angiotensin II could induce SIRT2 mediated microtubule reorganization in ECs [16]. Considering the pro-angiogenic roles of SIRT1 after MI and the homology between SIRT1 and SIRT2, we wanted to know whether SIRT2 could regulate reparative angiogenesis in MI.

Materials and methods

Establishment of MI model

All animal studies were performed in accordance with the Ethical Committee of the First Affiliated Hospital of Zhengzhou University, Zhengzhou University School of Medicine, and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. MI or sham-operation was performed according to published protocols [17]. Firstly, C57/BL6 male mice were anesthetized by intraperitoneal injection of Avertin (Sigma). Then the mice were intubated for assisted breathing after tracheotomy and an incision was made at the level of the left side of the third and fourth intercostal space under a surgical microscope. Finally, acute MI was induced by permanent ligation of the proximal left anterior descending coronary artery.

Isolation of cardiomyocytes, fibroblasts and ECs

The extraction of ECs, fibroblasts and cardiomyocytes from infarcted or sham-operated hearts was performed according to published literatures [17]. Briefly speaking, MI area and remote region from left ventricles (LVs) were harvested at 1, 3 and 14 days and digested with collagenase II. $CD146^+$ cells were then magnetic sorted by using a CD146 antibody (Miltenyi Biotech), and cardiomyocytes were obtained by sedimentation at room temperature for 10 minutes. As to the extraction of fibroblasts, tissues from MI area or remote region were cut into pieces and digested with liberase (Sigma) for 30 minutes. Then cells were plated in culture-flasks for 2 hours and adherent fibroblasts were obtained after discarding non-adherent cells.

Cell culture and lentiviral product

Human umbilical vein endothelial cells (HUVECs) were cultured in Dulbecco minimum essential medium (DMEM) containing 10% fetal bovine serum (FBS) at 37°C in a 5% CO_2-humidified incubator. Lentivirus expressing shSIRT2 and negative control shRNA were purchased from GenePharma, Shanghai. Cells were infected with lentivirus according to the manufacturer's protocol and the transfection efficiency was confirmed by quantitative real-time polymerase chain reaction (qRT-PCR) and western blot. The sequences of the shRNAs were established in **Table 1**.

Transient transfection

Commercialized pcDNA3.0-SIRT2 and control plasmids were purchased from Genomeditech, Shanghai. As to transient transfection, cells were seeded in 6-well plates and transfection was performed at 60% cell confluence with lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Cell proliferation and tubular formation assay were conducted 48 hours after transfection.

Quantitative real-time polymerase chain reaction

Total RNA was isolated from tissue or HUVECs with Trizol Reagent (Invitrogen, USA) according

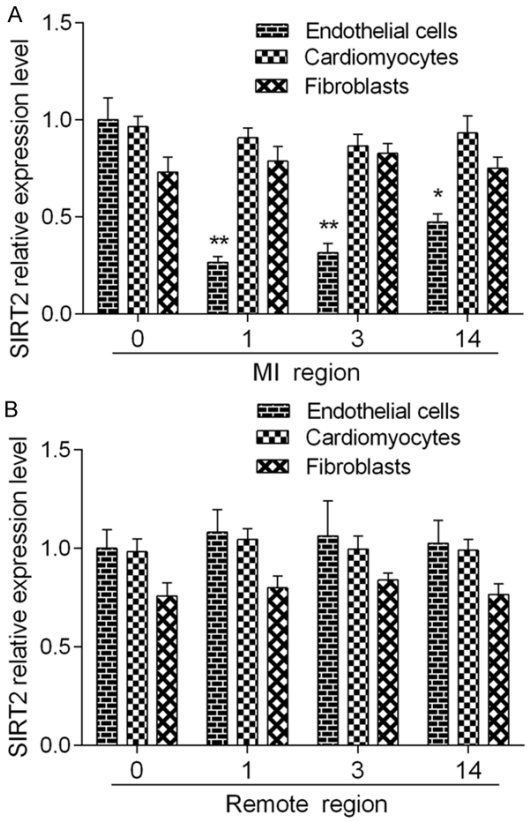

Figure 1. Selective SIRT2 down-regulation in endothelial cells after myocardial infarction (MI). Expression of SIRT2 relative to GAPDH in fractionated cardiomyocytes, fibroblasts and magnetic sorted endothelial cells ($CD146^+$ endothelial cells) of the peri-infarct (MI) or remote region were evaluated at 0, 1, 3, or 14 days after MI in mice. A, B. The expression level of SIRT2 was down-regulated in endothelial cells isolated from ischemic but not remote myocardium early after MI.

to the manufacturer's instructions. qRT-PCR was carried out on a 7900 HT Fast Real-Time PCR System (Applied Biosystems, USA) and the relative expression levels (fold change) of the target genes were determined by a $2^{-\Delta\Delta Ct}$ ($\Delta Ct = Ct^{sample} - Ct^{GAPDH}$; $\Delta\Delta Ct = \Delta Ct^{sample} - \Delta Ct^{reference}$) method. SIRT2 expression level of ECs in day 0 was considered as a calibrator and was normalized to 1. The primer sequences were listed in **Table 1**.

Evaluation of tube-like structures by matrigel assay

As to tubular formation assay, 96-well plates were coated with 50 μl matrigel (BD Bioscience) and polymerized at 37°C for 1 h. HUVECs were trypsinized and plated into 96-well plate (8×10^3 cells/well) coated with matrigel for 6 h. Then tubules were photographed by microscopy at 5 random fields and tubular numbers, length and intersections were counted by Image Pro Plus software.

Cell proliferation and migration assay

Cell Counting Kit-8 (Dojindo) was employed for cell proliferation assay. Briefly, cells (1.5×10^3/well) were seeded into 96-well culture plates in triplicate and incubated for 6 days at 37°C/5% CO_2 in a humidified incubator. Cell viability was examined at the same time of each day by microplate reader (Epoch, BioTek). As to cell migration analysis, 1×10^5 cells in 200 μl non-serum culture medium were placed in upper chamber of transwell (BD Biosciences) and 600 μl normal culture medium was placed in the lower chamber. After incubated for 48 h, migrated cells through transwell were fixed with methanol and stained with 1% crystal violet for statistics.

Immunoprecipitation and western blot

Protein G-agarose (Millipore) was employed for immunoprecipitation (IP). In brief, cell lysates were immunoprecipitated with 2 ug antibody overnight and incubated with 20 μl protein G-agarose for 4 h. After centrifugation, the precipitate was washed and boiled in the electrophoresis loading buffer for western blot assay. As to western blot assay, protein samples were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a PVDF membrane. After blocking with tris-buffered saline containing 0.1% Tween 20 (TBST) containing 5% BSA, the membranes were incubated with indicated primary antibodies and corresponding horseradish-peroxidase-conjugated secondary antibodies. The purpose bands were visualized by enhanced chemiluminescence (Pierce). The antibodies used in the present study were: SIRT2, β-catenin (abcam); GSK3β, AKT and the corresponding phosphorylation-specific-antibodies (Cell Signaling Technology); GAPDH (Santa Cruz). Anti-rabbit-HRP and anti-mouse-HRP antibodies (Millipore) were used as secondary antibodies.

Statistical analysis

SPSS 19.0 software (Chicago, SPSS inc, USA) was employed for statistical analysis and data was presented as means ± SD. Data between two groups were analyzed by using student's *t*

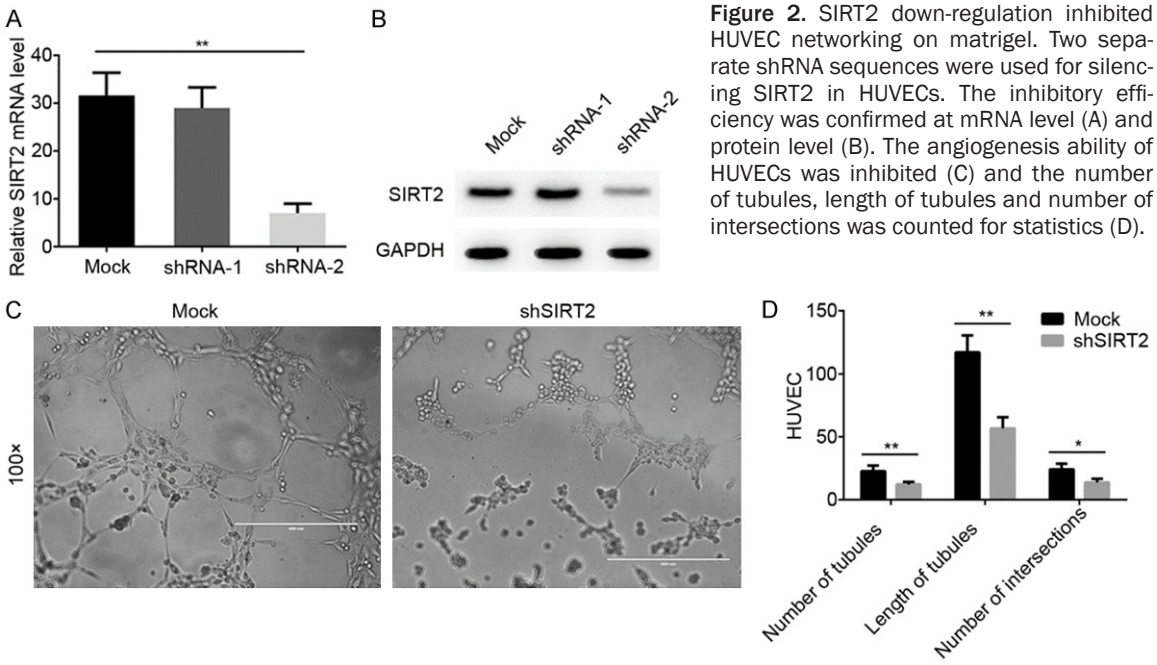

Figure 2. SIRT2 down-regulation inhibited HUVEC networking on matrigel. Two separate shRNA sequences were used for silencing SIRT2 in HUVECs. The inhibitory efficiency was confirmed at mRNA level (A) and protein level (B). The angiogenesis ability of HUVECs was inhibited (C) and the number of tubules, length of tubules and number of intersections was counted for statistics (D).

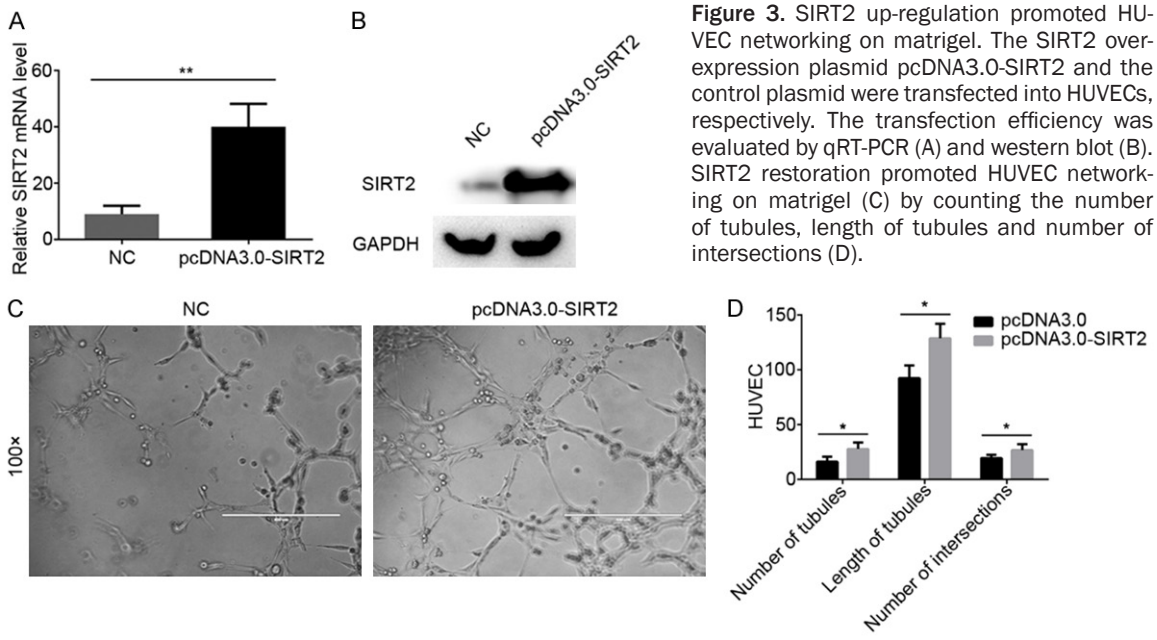

Figure 3. SIRT2 up-regulation promoted HUVEC networking on matrigel. The SIRT2 overexpression plasmid pcDNA3.0-SIRT2 and the control plasmid were transfected into HUVECs, respectively. The transfection efficiency was evaluated by qRT-PCR (A) and western blot (B). SIRT2 restoration promoted HUVEC networking on matrigel (C) by counting the number of tubules, length of tubules and number of intersections (D).

test. Data between three groups were analyzed by one-way ANOVA. *P* value less than 0.05 was considered to be statistically significant.

Results

SIRT2 is down-regulated in ECs by cardiac ischemia

To examine the expression levels of SIRT2 after MI, we extracted ECs, fibroblasts and cardiomyocytes from infarcted and sham-operated hearts, respectively. The mRNA and protein levels of SIRT2 in each cell type were evaluated, respectively. As shown in **Figure 1A** and **1B**, the mRNA level of SIRT2 was down-regulated in ECs isolated from ischemic but not remote myocardium early after MI.

SIRT2 regulated angiogenesis of HUVECs

We next evaluated whether SIRT2 could regulate the angiogenesis ability of HUVECs. HUVECs were transfected with shSIRT2 or the cor-

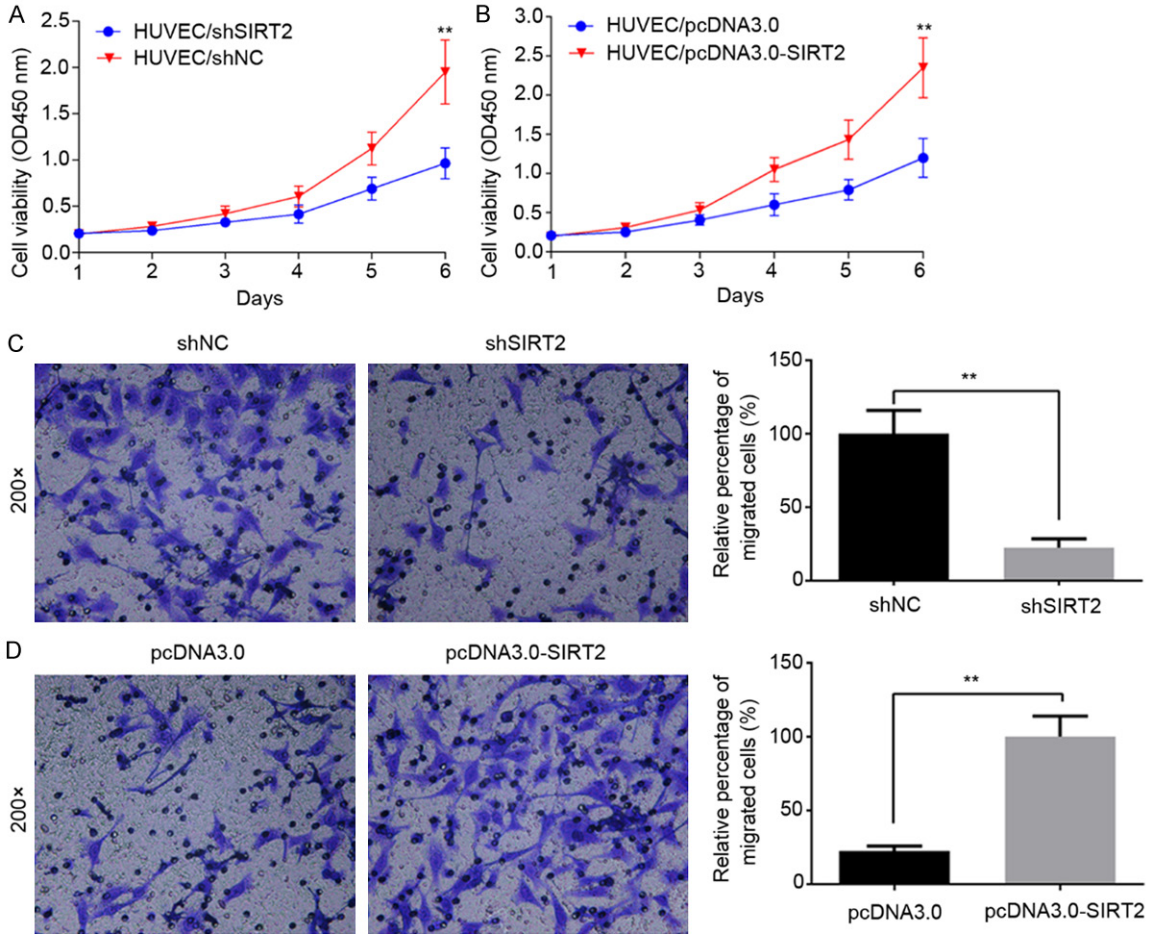

Figure 4. SIRT2 regulated proliferation and migration ability of HUVECs. Silencing SIRT2 inhibited HUVEC proliferation (A) while SIRT2 restoration promoted HUVEC proliferation (B) by CCK-8 analysis. Silencing SIRT2 decreased the migrated cells through transwell (C) while the migration ability of HUVECs yield a significant promotion after SIRT2 restoration (D).

responding negative control lentivirus. The transfection efficiency was evaluated by qRT-PCR and western blot, respectively (**Figure 2A** and **2B**). We observed that silencing SIRT2 could suppress the angiogenesis ability of HUVECs by counting the number of tubules, length of tubules and the number of intersections (**Figure 2C** and **2D**). To confirm these results, we transfected pcDNA3.0-SIRT2 or the control plasmid into HUVECs and the overexpression efficiency of SIRT2 was evaluated by qRT-PCR and western blot, respectively (**Figure 3A** and **3B**). As the result, overexpression of SIRT2 promoted the angiogenesis ability of HUVECs (**Figure 3C**) and the statistical result was presented in **Figure 3D**.

SIRT2 promoted HUVEC cell proliferation and migration

Published evidences indicated that the angiogenesis ability of HUVECs was accompany with its proliferation and migration abilities [18]. In the present study, we observed that shRNA targeting SIRT2 inhibited the proliferation ability of HUVECs, which was confirmed by the results of SIRT2 overexpression assay (**Figure 4A** and **4B**). The result of cell migration assay showed that knockdown of SIRT2 decreased the migrated HUVECs through transwell while overexpression of SIRT2 increased the migrated cells (**Figure 4C** and **4D**). Collectively, these results revealed that SIRT2 regulated proliferation and migration dependent angiogenesis of HUVECs.

SIRT2 regulated AKT signaling in HUVECs

We next attempted to reveal the mechanisms through which SIRT2 regulated angiogenesis. Published literatures had indicated that signaling pathways including AKT, STAT3, EGFR and YAP were participated in angiogenesis of MI or malignant tumors [19-22]. In the present work,

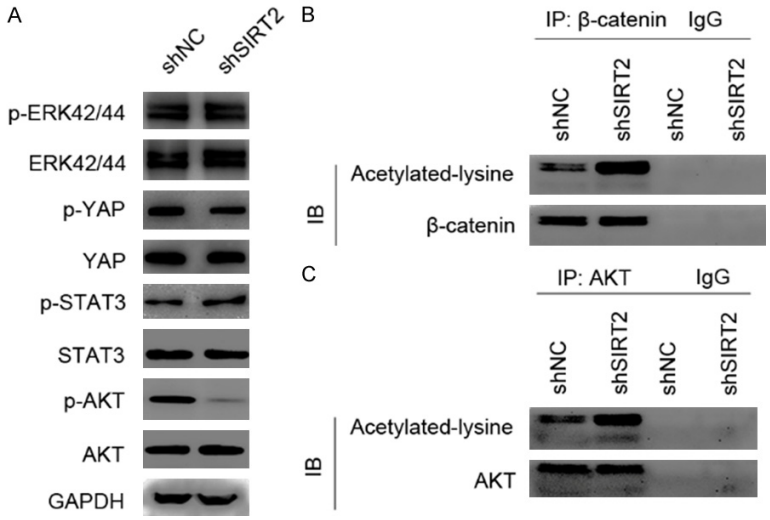

Figure 5. SIRT2 regulated Akt signaling in HUVECs. A. The levels of AKT, STAT3, EGFR, YAP and their corresponding phosphorylation proteins were evaluated after silencing SIRT2 and the level of phosphorylated AKT was significantly down-regulated. B, C. AKT and β-catenin were immunoprecipitated with corresponding antibodies and the acetylation level was observed respectively. Depletion of SIRT2 enhanced the acetylation level of AKT and β-catenin acetylation.

the level of phosphorylated AKT (activated AKT) was decreased after SIRT2 down-regulation, while the levels of STAT3, EGFR, YAP and their corresponding phosphorylation proteins didn't show any change (**Figure 5A**). Considering SIRT2 was a deacetylase, we next examined whether SIRT2 could regulate the acetylation level of AKT and its downstream effector β-catenin. Total cell lysates of shNC or shSIRT2 cells were immunoprecipitated with 2 ug AKT or β-catenin antibody respectively and detected by an anti-acetylated-lysine antibody. We observed a significant increase in the acetylation level of AKT and β-catenin after SIRT2 down-regulation (**Figure 5B** and **5C**).

Discussion

Angiogenesis, involving in degradation of vascular basement membrane, activation of vascular endothelial cell and reconstruction of new vascular network, is a complex process regulated by multiple molecules [23]. Functional angiogenesis is essential for cell proliferation and metastasis of human tumors [24]. As to cardiovascular system, cardiomyocyte growth and survival need glorious angiogenesis to provide nutrients. In addition, ECs/cardiomyocyte cross talk could protect cardiomyocytes from reperfusion injury after ischemia and control cardiomyocyte contractility [25]. Recently, reparative angiogenesis via transferring pro-angiogenic genes into the ischemic myocardium was proposed as a potential measurement for MI patients [26, 27]. The widely reported genes involved in angiogenesis or arteriogenesis include: (1) Growth factors like VEGF, FGF and Angiopoietin-1, which were expressed in ECs and monocytes, stimulating proliferation, migration, and tubular formation abilities. (2) Chemokines which controls the expression of key genes regulating angiogenesis. (3) Extracellular matrix proteins which could interact with integrins [28, 29]. Experimental evidences of these key factors proposed the promising therapeutic potential of reparative angiogenesis of MI patients.

In the present study, we found that SIRT2 was down-regulated in ECs isolated from ischemic but not remote myocardium early after MI. To explore whether the down-regulation of SIRT2 was functional or not, we transfected lentivirus expressing shSIRT2 and the negative control lentivirus into HUVECs to examine the effect of silencing SIRT2 on angiogenesis. Compared with the control group, shSIRT2 group yielded a significant decrease of tubular numbers, length of tubules and number of intersections. These results were further confirmed by SIRT2 overexpression assay in HUVECs. Moreover, knockdown of SIRT2 reduced migrated cells through transwell while overexpression of SIRT2 promoted the migration ability of HUVECs, which was in accordance with the results of tubular formation assay. These results indicated that SIRT2 promoted proliferation, migration and angiogenesis ability of HUVECs.

We next explored the signaling pathways underlying which SIRT2 regulated angiogenesis. Previously, other investigators had shown that signaling pathways including AKT, STAT3, EGFR and YAP were associated with reparative angiogenesis of MI [19-22]. Herein, we observed that

the phosphorylated AKT (activated AKT) was decreased after SIRT2 down-regulation, which was in accordance with the role of AKT in reparative angiogenesis reported by Emanueli et al [30]. Previous studies indicated that SIRT2 was a novel AKT binding partner and controlled AKT activation by regulating the acetylation level of AKT. In addition, considering SIRT2 was a NAD+ dependent deacetylase, we detected the acetylation level of AKT in HUVECS after silencing SIRT2. Interestingly, in consistent with a previously published article [31], acetylated AKT and β-catenin, which meant the change of AKT and β-catenin activity, was up-regulated after SIRT2 down-regulation, while more comprehensive studies were still needed. Taken together, these results provided a novel role of SIRT2 in the β-catenin signaling pathway by regulating AKT acetylation in HUVECs, which still need further studies.

In conclusion, we found a functional down-regulation of SIRT2 in a MI model. SIRT2 regulated angiogenesis of HUVECs via targeting AKT signaling pathway. SIRT2 might serve as a novel therapeutic target of MI patients.

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Disclosure of conflict of interest

None.

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References

[1] Steg PG, James SK, Atar D, Badano LP, Lundqvist CB, Borger MA, Di Mario C, Dickstein K, Ducrocq G and Fernandez-Aviles F. ESC guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. Eur Heart J 2012; 33: 2569-619.

[2] Thygesen K, Alpert JS, Jaffe AS, White HD, Simoons ML, Chaitman BR, Katus HA, Apple FS, Lindahl B and Morrow DA. Third universal definition of myocardial infarction. J Am Coll Cardiol 2012; 60: 1581-1598.

[3] Mehta SR, Yusuf S, Peters RJ, Bertrand ME, Lewis BS, Natarajan MK, Malmberg K, Rupprecht HJ, Zhao F and Chrolavicius S. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. Lancet 2001; 358: 527-533.

[4] Montalescot G, Wiviott SD, Braunwald E, Murphy SA, Gibson CM, McCabe CH, Antman EM; TRITON-TIMI 38 Investigators. Prasugrel compared with clopidogrel in patients undergoing percutaneous coronary intervention for ST-elevation myocardial infarction (TRITON-TIMI 38): double-blind, randomised controlled trial. Lancet 2009; 373: 723-731.

[5] Scheller B, Clever YP, Kelsch B, Hehrlein C, Bocksch W, Rutsch W, Haghi D, Dietz U, Speck U and Böhm M. Long-term follow-up after treatment of coronary in-stent restenosis with a paclitaxel-coated balloon catheter. JACC Cardiovasc Interv 2012; 5: 323-330.

[6] Yang Y, Shi C, Hou X, Zhao Y, Chen B, Tan B, Deng Z, Li Q, Liu J, Xiao Z, Miao Q and Dai J. Modified VEGF targets the ischemic myocardium and promotes functional recovery after myocardial infarction. J Control Release 2015; 213: 27-35.

[7] Iribarren C, Phelps BH, Darbinian JA, McCluskey ER, Quesenberry CP, Hytopoulos E, Vogelman JH and Orentreich N. Circulating angiopoietins-1 and -2, angiopoietin receptor Tie-2 and vascular endothelial growth factor-A as biomarkers of acute myocardial infarction: a prospective nested case-control study. BMC Cardiovasc Disord 2011; 11: 31.

[8] Zhao XY, Hu SJ, Li J, Mou Y, Chan CF, Jin J, Sun J and Zhu ZH. rAAV-mediated angiogenin gene transfer induces angiogenesis and modifies left ventricular remodeling in rats with myocardial infarction. J Mol Med (Berl) 2006; 84: 1033-1046.

[9] Michishita E, Park JY, Burneskis JM, Barrett JC and Horikawa I. Evolutionarily conserved and nonconserved cellular localizations and functions of human SIRT proteins. Mol Biol Cell 2005; 16: 4623-4635.

[10] Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM and Puigserver P. Nutrient control of glucose homeostasis through a complex of PGC-1α and SIRT1. Nature 2005; 434: 113-118.

[11] Purushotham A, Schug TT, Xu Q, Surapureddi S, Guo X and Li X. Hepatocyte-specific deletion of SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation. Cell Metab 2009; 9: 327-338.

[12] Gerhart-Hines Z, Rodgers JT, Bare O, Lerin C, Kim SH, Mostoslavsky R, Alt FW, Wu Z and Puigserver P. Metabolic control of muscle mito-

chondrial function and fatty acid oxidation through SIRT1/PGC-1α. EMBO J 2007; 26: 1913-1923.
[13] Samuel SM, Thirunavukkarasu M, Penumathsa SV, Paul D and Maulik N. Akt/FOXO3a/SIRT1-mediated cardioprotection by n-tyrosol against ischemic stress in rat in vivo model of myocardial infarction: switching gears toward survival and longevity. J Agric Food Chem 2008; 56: 9692-9698.
[14] Potente M, Ghaeni L, Baldessari D, Mostoslavsky R, Rossig L, Dequiedt F, Haendeler J, Mione M, Dejana E and Alt FW. SIRT1 controls endothelial angiogenic functions during vascular growth. Genes Dev 2007; 21: 2644-2658.
[15] Fukuda S, Kaga S, Zhan L, Bagchi D, Das DK, Bertelli A and Maulik N. Resveratrol ameliorates myocardial damage by inducing vascular endothelial growth factor-angiogenesis and tyrosine kinase receptor Flk-1. Cell Biochem Biophys 2006; 44: 43-49.
[16] Hashimoto-Komatsu A, Hirase T, Asaka M and Node K. Angiotensin II induces microtubule reorganization mediated by a deacetylase SIRT2 in endothelial cells. Hypertens Res 2011; 34: 949-956.
[17] Meloni M, Marchetti M, Garner K, Littlejohns B, Sala-Newby G, Xenophontos N, Floris I, Suleiman M-S, Madeddu P and Caporali A. Local inhibition of microRNA-24 improves reparative angiogenesis and left ventricle remodeling and function in mice with myocardial infarction. Mol Ther 2013; 21: 1390-1402.
[18] Seo DW, Li H, Guedez L, Wingfield PT, Diaz T, Salloum R, Wei BY and Stetler-Stevenson WG. TIMP-2 mediated inhibition of angiogenesis: an MMP-independent mechanism. Cell 2003; 114: 171-180.
[19] Siragusa M, Katare R, Meloni M, Damilano F, Hirsch E, Emanueli C and Madeddu P. Involvement of phosphoinositide 3-kinase gamma in angiogenesis and healing of experimental myocardial infarction in mice. Circ Res 2010; 106: 757-768.
[20] Xiao H, Bid HK, Jou D, Wu X, Yu W, Li C, Houghton PJ and Lin J. A novel small molecular STAT3 inhibitor, LY5, inhibits cell viability, cell migration, and angiogenesis in medulloblastoma cells. J Biol Chem 2015; 290: 3418-3429.
[21] Jin X, Yin J, Kim SH, Sohn YW, Beck S, Lim YC, Nam DH, Choi YJ and Kim H. EGFR-AKT-Smad signaling promotes formation of glioma stem-like cells and tumor angiogenesis by ID3-driven cytokine induction. Cancer Res 2011; 71: 7125-7134.
[22] Marti P, Stein C, Blumer T, Abraham Y, Dill MT, Pikiolek M, Orsini V, Jurisic G, Megel P, Makowska Z, Agarinis C, Tornillo L, Bouwmeester T, Ruffner H, Bauer A, Parker CN, Schmelzle T, Terracciano LM, Heim MH and Tchorz JS. YAP promotes proliferation, chemoresistance, and angiogenesis in human cholangiocarcinoma through TEAD transcription factors. Hepatology 2015; 62: 1497-1510.
[23] Carmeliet P and Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature 2011; 473: 298-307.
[24] Weis SM and Cheresh DA. Tumor angiogenesis: molecular pathways and therapeutic targets. Nat Med 2011; 17: 1359-1370.
[25] Cochain C, Channon KM and Silvestre JS. Angiogenesis in the infarcted myocardium. Antioxid Redox Signal 2013; 18: 1100-1113.
[26] Siragusa M, Katare R, Meloni M, Damilano F, Hirsch E, Emanueli C and Madeddu P. Involvement of phosphoinositide 3-kinase γ in angiogenesis and healing of experimental myocardial infarction in mice. Circ Res 2010; 106: 757-768.
[27] Al Haj Zen A, Oikawa A, Bazan-Peregrino M, Meloni M, Emanueli C, Madeddu P. Inhibition of delta-like-4-mediated signaling impairs reparative angiogenesis after ischemia. Circ Res 2010; 107: 283-293.
[28] Shibuya M. Vascular endothelial growth factor and its receptor system: physiological functions in angiogenesis and pathological roles in various diseases. J Biochem 2013; 153: 13-19.
[29] Kumar S, Shahabuddin S, Haboubi N, West D, Arnold F, Reid H and Carr T. Angiogenesis factor from human myocardial infarcts. Lancet 1983; 322: 364-368.
[30] Emanueli C, Salis MB, Pinna A, Graiani G, Manni L and Madeddu P. Nerve growth factor promotes angiogenesis and arteriogenesis in ischemic hindlimbs. Circulation 2002; 106: 2257-2262.
[31] Chen J, Chan AW, To KF, Chen W, Zhang Z, Ren J, Song C, Cheung YS, Lai P and Cheng SH. SIRT2 overexpression in hepatocellular carcinoma mediates epithelial to mesenchymal transition by protein kinase B/glycogen synthase kinase-3β/β-catenin signaling. Hepatology 2013; 57: 2287-2298.