KDM3A is a Positive Regulator of Cardiac Fibroblasts Conversion that Increases Smad3 Phosphorylation Following TGF β 1 Stimulation

Xiaopei Liu

Renmin Hospital of Wuhan University: Wuhan University Renmin Hospital

Zhou Jining

Renmin Hospital of Wuhan University: Wuhan University Renmin Hospital

Bofang Zhang

Renmin Hospital of Wuhan University: Wuhan University Renmin Hospital

Gen Liu

Renmin Hospital of Wuhan University: Wuhan University Renmin Hospital

Qi Hu

Renmin Hospital of Wuhan University: Wuhan University Renmin Hospital

Jing Chen (✉ chenjing1982@whu.edu.cn)

Wuhan University Renmin Hospital <https://orcid.org/0000-0002-4037-7158>

Research Article

Keywords: KDM3A, Smad3, fibroblast-to-myofibroblast transition, cardiac fibrosis

Posted Date: November 19th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1060401/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The epigenetic molecule KDM3A has been shown to be involved in improving cardiovascular diseases, but its effect on cardiac fibroblasts (CFs) remains unclear. Thus, we designed the gain- and loss-of-function experiments to investigate the biological functions of KDM3A in cardiac fibroblasts (CFs). Moreover, we added a SIS3-HCL (a specific inhibitor of p-Smad3) to explore the underlying mechanism. The cells viability and migration were verified by CCK-8 and cell migration experiments, respectively, and the degree of fibrosis was measured by Western blot analysis. Our data reveal that KDM3A enhance the proliferation and migration of cardiac fibroblasts, meanwhile, increasing the fibroblast-to-myofibroblast transition, while enabling Smad3 phosphorylation response to TGF β 1 stimuli. However, these results could be abolished by SIS3-HCL, an inhibitor of the p-Smad3. Furthermore, KDM3A inhibition obviously protect cardiac fibroblasts conversion against TGF β 1 stimuli. These results identify that KDM3A may be a novel regulator of the cardiac fibroblasts conversion, through its ability to modulate phosphorylation of Smad3 following TGF β 1 stimuli.

1. Introduction

Cardiac fibrosis is involved in the pathogenesis of most myocardial diseases^[1]. Despite tissue homeostasis requires the maintenance of collagen fibers, sustained chronic injury and microenvironmental perturbation will reverse the benign tissue repair into unrestrained or dysregulated fibrotic scar, which can eventually lead to myocardial structural and pathophysiological alterations and heart failure^[2, 3]. Cardiac fibroblasts (CFs) are responsible for extracellular matrix (ECM) homeostasis. Excessive ECM deposition can further active resident cardiac fibroblasts differentiate into myofibroblast, which in turn exacerbate secretion of ECM^[4]. There are multiple mechanistic pathways involved in the conversion of myocardial fibroblasts into myofibroblasts (CMF). However, efficacious therapies to prevent excessive cardiac fibrosis are lacking. Therefore, identifying novel options are needed.

KDM3A, also known as JMJD2A, plays a role as a histone methyltransferase by demethylating mono- or dimethylated H3K9, has a close relationship of myocardial tissue repair and remodeling, partially through maintaining inflammation, Foxidative stress, and neovascularization^[5, 6]. Previous study shows that KDM3A stimulation could exacerbate pathological fibrosis in cardiac hypertrophy ^[7], however, the role of KDM3A and its underlying mechanism in CFs has never been addressed thoroughly. This present study was undertaken to explore the cardiac fibroblast differentiation induced with TGF β 1 by KDM3A administration or inhibition. Further, we determined investigated the molecular mechanism.

2. Materials And Methods

2.1 Adenovirus construction and transfection

The adenovirus that encoding the full-length rat KDM3A sequence (AdKDM3A) and KDM3A-siRNA (AdshKDM3A) were validated to significantly regulate the protein expression of KDM3A in previous

studies^[5, 6, 8]. And western blots for protein expression analysis were implemented to determine whether the adenovirus was successfully transfected in these experiments.

2.2 The culture and identification of CFs

Primary cardiac fibroblasts were isolated from neonatal Sprague-Dawley (SD) (n=10, 1-3 days old) rats according to the differential centrifugation method as previously described^[9]. CFs were routinely at passages 3-4 for subsequent experiments.

The cell purity was analyzed by anti-vimentin (Abcam) and anti- α -SMA (MilliporeSigma) antibodies in an immunofluorescence containing experiment. The ratios of α -SMA and Vimentin staining were observed under a fluorescence microscope to identify the purity of CFs, and cells that negative for α -SMA and 95% positive for Vimentin were used for the subsequent experiments^[10, 11].

2.3 Experimental Design

For the loss-of-function experiments, CFs were randomly classified into 4 groups (n=3/group) in the following order: 1) Control + AdshRNA group: transfected by siRNA group; 2) Control + AdshKDM3A group: transfected by siRNA-KDM3A group; 3) TGF- β 1 + AdshRNA group: transfected by siRNA in the response to TGF- β 1; 4) TGF- β 1 + AdshKDM3A group: transfected by siRNA-KDM3A in the response to TGF- β 1. We further investigated KDM3A gain-of-function experiments, the experimental group were the same as the loss-of-function experiments, but in order to explore the underlying mechanism, we added a SIS3-HCL (a specific inhibitor of p-Smad3) (Selleck) as previous described^[12] in TGF- β 1 + AdshKDM3A group as another group. Next, the cells viability and migration were verified by CCK-8 and cell migration experiments, respectively, and the degree of fibrosis was measured by Western blot analysis.

2.4 Immunofluorescence staining

To evaluate the degree of cardiac fibrosis, we performed α -SMA and Vimentin-specific immunofluorescence staining of CFs. According to a previous study^[13], CFs were harvested, fixed with 4% paraformaldehyde, and blocked with 3% goat serum for 1 h. Subsequently, anti- α -SMA and anti-vimentin primary antibodies were applied to the cells, followed by Alexa Fluor-conjugated secondary antibodies. Nuclear staining was achieved using DAPI. The cells were visualized and photographed under a fluorescence microscope.

2.5 CCK-8 assay

Cardiac fibroblast proliferation was analyzed using the CCK-8 assay as described in detail previously^[10]. Briefly, CFs were digested and reseeded in a 96-well plate at a density of 1×10^3 cells per well, 100 μ l of medium was added, and the cells were cultured for 48 h. Ten microliters of CCK-8 reagent (Dojindo Molecular Technologies) was added to each well and incubated for 4 hours at 37°C. Finally, the optical density (OD) values were measured at 450 nm using a microplate reader.

2.6 Cell migration assay

Cell migration was determined using a 24-well Transwell plate (Corning Costar). Briefly, CFs were resuspended in the upper chamber of the plate with serum-starved. And culture medium containing 10% FBS was added to the lower chamber as a chemoattractant, then incubation at 37 °C for 24 h. The migrated cells were counted in five randomly chosen fields at magnification of 200× by light microscopy (Olympus Corporation, Tokyo, Japan), and analysis by Image J 1.51w software.

2.7 Western blot analysis

After CFs were harvested, total protein was extracted, and the protein concentration was measured using a BCA kit according to the manufacturer's instructions. Equal amounts of protein were subjected to SDS-PAGE and transferred to polyvinylidene difluoride membranes. The membranes were incubated with the appropriate primary antibodies and then probed with peroxidase-conjugated secondary antibodies for 2 h at room temperature. Finally, the results were analyzed with an ECL detection system, the bands were visualized with ImageLab 5.2 software, and the intensities were normalized to GAPDH. Antibodies against Collagen I, α -SMA, CTGF vimentin, DDR2, KDM3A and GAPDH were obtained from Abcam. Antibodies against Smad3 and p-Smad3 were obtained from CST.

2.8 Statistical analysis

Statistical analyses were performed using GraphPad Prism 7.0 software (GraphPad Software Inc, USA). All data were expressed as the mean $\pm$ standard deviation (SD). Intergroup differences were compared using ANOVA. Statistical significance was set at $p < 0.05$.

3. Results

3.1 KDM3A expression in CFs.

Firstly, we identified the phenotype and purity of CFs after 2-3 generations. Previous studies demonstrated that vimentin was surface-specific antigen of cardiac fibroblasts^[14], and α -SMA antibodies was surface marker of myofibroblasts^[15]. As shown in Figure 1a, we successfully isolated highly purified primary rat CFs by immunofluorescence analysis. Ninety-five percent of cells were stained with vimentin

(red), almost no cells were stained with α -SMA (green), indicating that the isolated CFs could be utilized for subsequent experiments.

Next, we constructed relevant adenovirus to administered KDM3A expression. CFs were infected with KDM3A(AdKDM3A) or siRNA-KDM3A(AdshKDM3A). The results showed CFs transfected with AdKDM3A or AdshKDM3A (Figure 1b) emitted strong green fluorescence (GFP), indicating that target virus vectors were successfully transfected into the CFs. Then, we further measured the protein expression of KDM3A by Western blot analysis, and the results indicated that KDM3A expression was significantly enhanced after transfection with AdKDM3A, while AdshKDM3A transfection blocked KDM3A expression (Figure 1c-d). These data indicated that the target adenovirus we constructed effectively regulated the protein expression of KDM3A in CFs.

3.2 TGF β 1 induces cardiac fibroblast differentiation and significantly impacts KDM3A expression.

As we all know, TGF β /Smad signaling as a classic profibrotic pathway that increase fibroblast abundance and cardiac fibroblast differentiation is a key event in cardiac remodeling^[16, 17]. Moreover, TGF β 1 is the most common subtype of TGF β . Thus, firstly, we verified the effects of TGF β 1 on cardiac fibroblast differentiation, and expression levels of vimentin, DDR2, α -SMA and CTGF were used as standard indexes of cardiac fibroblast differentiation^[18]. Figure 2a-b showed that CTGF and α -SMA were significantly upregulated, while Vimentin and DDR2 were significantly downregulated response to TGF β 1 treatment for 24 h. As expected, we demonstrated that TGF β 1 could induce cardiac fibroblast differentiation. Importantly, consistent with the previous researches, KDM3A might participated in cardiac fibrosis^[19]. In present study, we found that compared to control group, KDM3A level was significantly elevated (Figure 2c-d). Taken together, these results suggest that TGF- β 1 promotes cardiac fibroblast differentiation and significantly enriches KDM3A expression. Based on the above observations, we speculated that KDM3A might be a key regulation factor during the course of cardiac fibroblast differentiation. To manifest this hypothesis, KDM3A loss- and gain- of- function assays were preformed in CFs.

3.3 KDM3A disruption significantly influences CFs proliferation and migration.

Based on above observations, we further explored whether KDM3A can intervene in specific biological functions of fibroblasts. First, we designed CCK-8 and migration experiments to evaluate fibroblast proliferation and migration function, respectively. The results showed that loss of KDM3A markedly suppressed CFs proliferation and migration under the stimulation of TGF- β 1 for 24 h. (Figure 3a, c, d); however, when KDM3A was upregulated, the opposite tendency was observed, CFs proliferation and

migration were significantly increased (3b, c, d). Collectively, suppression of KDM3A in CFs could abrogate the proliferation and migration, but overexpression of KDM3A had the opposite consequences.

3.4 KDM3A is a positive regulator of cardiac fibroblast differentiation, but KDM3A inhibition can reverse myofibroblast conversion.

To determine whether KDM3A is involved in cardiac fibroblast differentiation, we analyzed the changes in α -SMA, collagen I, and CTGF expression in response to changes in KDM3A expression. CFs were transfected with siRNA-KDM3A to knock down its expression, and transfected with AdKDM3A to active its expression. The results showed that CFs conversion was decreased by KDM3A downregulation that the expression of collagen I, CTGF and α -SMA in fibroblast was significantly lower than that in the control group under the stimulation of TGF- β 1 for 24 h (Figure 4a). Consistent with the previous studies, after KDM3A upregulation, the conversion of CFs was obviously aggravated (Figure 4b). These data indicate that KDM3A inhibition could protect CFs against the conversion, but overexpression of KDM3A could further exacerbate CFs differentiation.

3.5 KDM3A regulates the biological function of CFs through the Smad3-dependent manner.

It is obviously noted that cardiac fibroblast differentiation is pivotal for the pathogenesis of cardiac fibrosis and hypertrophy^[11, 20]. However, the underlying molecular mechanisms of cardiac fibroblast differentiation are still confused. Since CFs were featured with an enhanced expression of KDM3A induced by TGF- β 1 for 24 h (Figure 2c), we further illustrated KDM3A participate in regulating functionalities of CFs (Figure 3). Additionally, we found that high expression of KDM3A could also simultaneously increase Smad3 phosphorylation, and Smad3 level had no significant change (Figure 4d). Nevertheless, after knocked down KDM3A by transfected siRNA-KDM3A, Smad3 phosphorylation was suppressed evidently by treated with TGF- β 1 (Figure 4c). These findings prompted us to assume that KDM3A may be induced by TGF- β 1 signaling, and then regulating functionalities of CFs by Smad3 signaling.

To confirm this hypothesis, CFs were treated with SIS3-HCL (a specific inhibitor of p-Smad3), followed by TGF- β 1 induction. It was remarkably noted that p-Smad3 deficiency by SIS3-HCL could both significantly abolish KDM3A positive effect, which was accompanied with weakened cardiac fibroblast differentiation related proteins (collagen I, CTGF, α -SMA) induced by TGF- β 1 (Figure 4b, d), indicating that Smad3 were indispensable factors for KDM3A alter cardiac fibroblast differentiation in response to TGF- β 1. Moreover, consistent with the results of cardiac fibroblast differentiation, we found that p-Smad3 inhibition by SIS3-HCL was indeed impair CFs proliferation and migration ability (Figure 3 b,c,d). Altogether, these data

corroborated that overexpression of KDM3A aggravated cardiac fibroblast differentiation induced by TGF- β 1 was a Smad3-dependent manner.

4. Discussion

In the present study, we demonstrated a novel function for the first time that KDM3A increases cardiac fibroblast differentiation, improves CFs migration and proliferation in response to TGF- β 1 stimulation. We also observed that these positive effects were suppressed by the p-Smad3 inhibitor, which may be a compensatory response against cardiac fibrosis. By contrast, KDM3A deficiency could reverse these effects, protect CFs from TGF- β 1 induced cardiac fibroblast differentiation. These results further suggest that KDM3A exhibits its pro-fibrotic effects at least partly be relieved by suppressing Smad3 signaling pathway in response to TGF- β 1 stimulation. Consistently, KDM3A deficiency may contribute to its ability to prevent collagen deposition and cardiac fibrosis. These findings may open a new horizon in cardiac fibrosis disease therapy.

The accumulation of collagen fibers is widespread in the myocardial interstitium of most chronic heart diseases^[21]. Cardiac remodeling caused by myocardial fibrosis will eventually become a decisive factor for heart failure and its clinical progression, importantly, the more interstitial fibrosis deposited, the more likely it is to increase the mortality of patients^[22]. Thus, it is urgent need to explore strategies to inhibit cardiac fibroblasts proliferate and phenotype conversion which were critical evolution of cardiac fibrosis. In the current study, we observed KDM3A elevate TGF- β 1 induced CFs migration, proliferation and cardiac fibroblast differentiation, but suppressed by p-Smad3 inhibitor, indicating that KDM3A may generate pro-fibrotic effects by Smad3 signaling in TGF- β 1 stimuli. Thus, the involvement of the TGF- β 1/KDM3A/Smad3 axis may partly perform the differentiation of fibroblasts into myofibroblasts. However, the more complex and warrants in these pathological processes need further investigation.

While the recent studies of KDM3A provide a powerful evidence for the investigation of the complicated pathogenesis of cardiovascular diseases and molecular mechanisms involved^[5, 8, 19], the role of KDM3A in the regulation of cardiac fibrosis and functions remain elusive. The Gene Expression Omnibus (GEO) database revealed that KDM3A can target TGF- β 1, thereby regulating the TGF- β 1/Smad signaling pathway and affecting the progression of fibrosis^[23]. Previous studies targeting cardiac resident cells, such as cardiomyocyte, endothelial cells and macrophages, KDM3A have generated several controversial results. Depletion of KDM3A in endothelial cells dampened vascular inflammation by inhibiting CSF1 transcription and macrophage recruitment^[24]. However, by contrast, our previous study demonstrated that KDM3A improved cardiac performance and alleviated cardiac fibrosis in MI-induced myocardial injury by mitigating myocardial apoptosis^[25]. Perhaps, we speculate that the different effects of KDM3A in cardiovascular diseases may vary depending on diverse pathological conditions. In general, KDM3A has potential myocardial damage effects following cardiac hypertrophy through both pro-fibrosis and pro-inflammation mechanisms^[26-28].

In addition, previous studies have shown that crosstalk between Brg1 and KDM3A via co-regulate pro-cholesterogenic transcription to contribute to SREBP2-dependent cholesterol synthesis in hepatocyte^[29]. Interestingly, excessive synthesis of cholesterol may become the pathological basis of cardiac hypertrophy and atherosclerosis. Thus, these results not only raise novel insights into the understanding of the role of KDM3A underlying cardiac hypertrophy, but also provide a potent amenable therapeutic approach to improve myocardial fibrosis induced cardiac hypertrophy. Importantly, KDM3A, a ubiquitous histone lysine demethylase, functions as epigenetic modifications in regulating tissue fibrosis^[19, 30]. Indeed, emerging evidence has demonstrated that KDM3A silencing equivalently attenuated HG-induced CTGF induction in renal tubular epithelial cells by decreasing dimethylated H3K9 recruit to the CTGF promoter to suppress transcription^[30]. Unfortunately, following above studies only confirmed KDM3A participate in modulating myocardial fibrosis in animal models. Therefore, we explored whether KDM3A exerts a similar pro-fibrotic effect directly in TGFβ1-stimulated CFs in vitro. Consistently, the current study indicated that KDM3A significantly induced CFs migration, proliferation and cardiac fibroblast differentiation, which was attenuated by p-Smad3 inhibitor treatment.

TGFβ1 is a recognized fibrogenic cytokine, which bind to TGFβR1 receptor before phosphorylated by TGFβR2, and then induce canonical pathway (mainly Smad2/3 proteins), leading to collagen production^[31, 32]. Interestingly, in this study, KDM3A knockdown in primary CFs resulted in significant down-regulation of pro-fibrotic genes and predominantly decreased Smad3 phosphorylation in response to TGFβ1. Moreover, KDM3A was upregulated by TGF-β1 stimuli. Collectively, KDM3A may act as a mediator between TGF-β1 and Smad3, and jointly mediate the pathological process of myocardial fibrosis. Supporting these findings, KDM3A was robustly delayed renal fibrosis degree via elevate Smad2/3 phosphorylation^[33]. Accumulating evidence indicates that Smad3 is a key intracellular regulatory protein in the TGF-β superfamily signal cascade^[17]. On the one hand, Smad3 is involved in regulation of cardiac fibroblast phenotype and function^[34]. On the other hand, Smad3 has been suggested to promote the transformation of cardiac fibroblasts into myofibroblasts, then inhibiting protease-mediated ECM degradation to maintain a stable microenvironment, thus, affecting the formation of tissue scars^[35, 36]. Besides, attenuation of Smad3 phosphorylation and disturbing SMAD3/SMAD4 complex formation obviously abrogated expression of hepatic XPO4, which associates with severity of fibrosis in metabolic associated fatty liver disease^[37]. In keeping with an expanding body of evidence, we found that cardiac fibroblast phenotype and function were significantly modulated in the KDM3A activation condition, accompanied with Smad3 phosphorylation increases. Additionally, the available results suggested that Overexpression KDM3A in cardiomyocytes obviously promoted the transcription of the fibrosis-related factor Timp1, and further exacerbating the pathological progression of cardiac fibrosis and ventricular remodeling^[7]. This view coincides with the therapeutic mechanism of KDM3A, which corporately shows that it plays a multi-channel regulatory role in cardiac fibrosis, but targets the Smad3 signaling pathway in the present study.

5. Conclusion

In summary, we characterized KDM3A as a potential bifurcation point in the phenotypic transition of fibroblasts to myofibroblasts in response to TGF β 1 stimulation and, to our knowledge, were the first focus on cardiac fibroblasts. Pathophysiologically, we highlight the complexity of the molecular mechanisms of KDM3A in cardiac fibrosis, providing data to illustrate that KDM3A might influence cardiac fibroblasts proliferation, migration and conversion by manipulating the Smad3-dependent pathway.

Declarations

Acknowledge

This study was supported by the National Natural Science Foundation of China (81570331) and Nature Science Foundation of Hubei Province(2018CFB240).

Compliance with Ethical Standards

Funding: This study was supported by the National Natural Science Foundation of China (81570331) and Nature Science Foundation of Hubei Province(2018CFB240).

Ethical approval:

All rats in this study were all supplied from the Medical Laboratory Animal Center of Wuhan University. The study and animal care protocols were approved by the Animal Care and Use Committee of Wuhan University and conformed to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Conflict of Interest:

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and company that could be construed as influencing the position presented in the manuscript entitled “KDM3A is a positive regulator of cardiac fibroblasts conversion that increases Smad3 phosphorylation following TGF β 1 stimulation”.

References

1. Kong P, Christia P, Frangogiannis NG (2014) The pathogenesis of cardiac fibrosis[J]. *Cell Mol Life Sci* 71(4):549–574
2. Sutton MG, Sharpe N (2000) Left ventricular remodeling after myocardial infarction: pathophysiology and therapy[J]. *Circulation* 101(25):2981–2988

3. Souders CA, Bowers SL, Baudino TA (2009) Cardiac fibroblast: the renaissance cell[J]. *Circ Res* 105(12):1164–1176
4. Gibb AA, Lazaropoulos MP, Elrod JW (2020) Myofibroblasts and Fibrosis: Mitochondrial and Metabolic Control of Cellular Differentiation[J]. *Circ Res* 127(3):427–447
5. Zhang BF, Jiang H, Chen J et al (2020) LncRNA H19 ameliorates myocardial infarction-induced myocardial injury and maladaptive cardiac remodelling by regulating KDM3A[J]. *J Cell Mol Med* 24(1):1099–1115
6. Chen J, Zhang J, Yang J et al (2017) Histone demethylase KDM3a, a novel regulator of vascular smooth muscle cells, controls vascular neointimal hyperplasia in diabetic rats[J]. *Atherosclerosis* 257:152–163
7. Zhang QJ, Tran T, Wang M et al (2018) Histone lysine dimethyl-demethylase KDM3A controls pathological cardiac hypertrophy and fibrosis[J]. *Nat Commun* 9(1):5230
8. Liu X, Chen J, Zhang B et al (2019) KDM3A inhibition modulates macrophage polarization to aggravate post-MI injuries and accelerates adverse ventricular remodeling via an IRF4 signaling pathway[J]. *Cell Signal* 64:109415
9. Martin R, Gutierrez B, Cordova C et al (2020) Secreted Phospholipase A2-IIA Modulates Transdifferentiation of Cardiac Fibroblast through EGFR Transactivation: An Inflammation-Fibrosis Link[J]. *Cells*, 9(2)
10. Zhou Y, Shiok TC, Richards AM et al (2018) MicroRNA-101a suppresses fibrotic programming in isolated cardiac fibroblasts and in vivo fibrosis following trans-aortic constriction[J]. *J Mol Cell Cardiol* 121:266–276
11. Rodriguez P, Sassi Y, Troncone L et al (2019) Deletion of delta-like 1 homologue accelerates fibroblast-myofibroblast differentiation and induces myocardial fibrosis[J]. *Eur Heart J* 40(12):967–978
12. Wu H, Wang D, Shi H et al (2021) PM2.5 and water-soluble components induce airway fibrosis through TGF-beta1/Smad3 signaling pathway in asthmatic rats[J]. *Mol Immunol* 137:1–10
13. Zong J, Zhang H, Li FF et al (2018) NLRP1 promotes TGF-beta1-induced myofibroblast differentiation in neonatal rat cardiac fibroblasts[J]. *J Mol Histol* 49(5):509–518
14. Rodriguez P, Sassi Y, Troncone L et al (2019) Deletion of delta-like 1 homologue accelerates fibroblast-myofibroblast differentiation and induces myocardial fibrosis[J]. *Eur Heart J* 40(12):967–978
15. Tarbit E, Singh I, Peart JN et al (2019) Biomarkers for the identification of cardiac fibroblast and myofibroblast cells[J]. *Heart Fail Rev* 24(1):1–15
16. Meng XM, Nikolic-Paterson DJ, Lan HY (2016) TGF-beta: the master regulator of fibrosis[J]. *Nat Rev Nephrol* 12(6):325–338
17. Hanna A, Humeres C, Frangogiannis NG (2021) The role of Smad signaling cascades in cardiac fibrosis[J]. *Cell Signal* 77:109826

18. Zhou XL, Xu H, Liu ZB et al (2018) miR-21 promotes cardiac fibroblast-to-myofibroblast transformation and myocardial fibrosis by targeting Jagged1[J]. *J Cell Mol Med*,
19. Zhang QJ, Tran T, Wang M et al (2018) Histone lysine dimethyl-demethylase KDM3A controls pathological cardiac hypertrophy and fibrosis[J]. *Nat Commun* 9(1):5230
20. Zhang QJ, Tran T, Wang M et al (2018) Histone lysine dimethyl-demethylase KDM3A controls pathological cardiac hypertrophy and fibrosis[J]. *Nat Commun* 9(1):5230
21. Lopez B, Ravassa S, Moreno MU et al (2021) Diffuse myocardial fibrosis: mechanisms, diagnosis and therapeutic approaches[J]. *Nat Rev Cardiol* 18(7):479–498
22. Frangogiannis NG (2019) Cardiac fibrosis: Cell biological mechanisms, molecular pathways and therapeutic opportunities[J]. *Mol Aspects Med* 65:70–99
23. Ding H, Xu Y, Jiang N (2020) Upregulation of miR-101a Suppresses Chronic Renal Fibrosis by Regulating KDM3A via Blockade of the YAP-TGF-beta-Smad Signaling Pathway[J]. *Mol Ther Nucleic Acids* 19:1276–1289
24. Zhang X, Liu S, Weng X et al (2018) Brg1 trans-activates endothelium-derived colony stimulating factor to promote calcium chloride induced abdominal aortic aneurysm in mice[J]. *J Mol Cell Cardiol* 125:6–17
25. Zhang BF, Jiang H, Chen J et al (2020) LncRNA H19 ameliorates myocardial infarction-induced myocardial injury and maladaptive cardiac remodelling by regulating KDM3A[J]. *J Cell Mol Med* 24(1):1099–1115
26. Ding H, Xu Y, Jiang N (2020) Upregulation of miR-101a Suppresses Chronic Renal Fibrosis by Regulating KDM3A via Blockade of the YAP-TGF-beta-Smad Signaling Pathway[J]. *Mol Ther Nucleic Acids* 19:1276–1289
27. Li Z, Zhang Y, Zhang Y et al (2020) BRG1 Stimulates Endothelial Derived Alarmin MRP8 to Promote Macrophage Infiltration in an Animal Model of Cardiac Hypertrophy[J]. *Front Cell Dev Biol* 8:569
28. Liu L, Zhao Q, Kong M et al (2021) Myocardin-related transcription factor A (MRTF-A) regulates integrin beta 2 transcription to promote macrophage infiltration and cardiac hypertrophy in mice[J]. *Cardiovasc Res*,
29. Fan Z, Kong M, Li M et al (2020) Brahma Related Gene 1 (Brg1) Regulates Cellular Cholesterol Synthesis by Acting as a Co-factor for SREBP2[J]. *Front Cell Dev Biol* 8:259
30. Shao J, Xu H, Wu X et al (2020) Epigenetic activation of CTGF transcription by high glucose in renal tubular epithelial cells is mediated by myocardin-related transcription factor A[J]. *Cell Tissue Res* 379(3):549–559
31. Lee TH, Yeh CF, Lee YT et al (2020) Fibroblast-enriched endoplasmic reticulum protein TXNDC5 promotes pulmonary fibrosis by augmenting TGFbeta signaling through TGFBR1 stabilization[J]. *Nat Commun* 11(1):4254
32. Derynck R, Zhang YE (2003) Smad-dependent and Smad-independent pathways in TGF-beta family signalling[J]. *Nature* 425(6958):577–584

33. Chang L, Wang Q, Ju J et al (2020) Magnoflorine Ameliorates Inflammation and Fibrosis in Rats With Diabetic Nephropathy by Mediating the Stability of Lysine-Specific Demethylase 3A[J]. *Front Physiol* 11:580406
34. Dobaczewski M, Bujak M, Li N et al (2010) Smad3 signaling critically regulates fibroblast phenotype and function in healing myocardial infarction[J]. *Circ Res* 107(3):418–428
35. Hu HH, Chen DQ, Wang YN et al (2018) New insights into TGF-beta/Smad signaling in tissue fibrosis[J]. *Chem Biol Interact* 292:76–83
36. Morine KJ, Qiao X, York S et al (2018) Bone Morphogenetic Protein 9 Reduces Cardiac Fibrosis and Improves Cardiac Function in Heart Failure[J]. *Circulation* 138(5):513–526
37. Metwally M, Bayoumi A, Khan A et al (2021) Copy number variation and expression of exportin-4 associates with severity of fibrosis in metabolic associated fatty liver disease[J]. *EBioMedicine* 70:103521

Figures

Figure 1

Identification of cardiac fibroblasts and transfection of AdKDM3A or AdshKDM3A adenoviruses. a Immunofluorescence staining of vimentin (red) and α -SMA (green) ($\times 200$). The cell nuclei were stained with a DAPI kit; b: Representative fluorescent images of adenovirus transfection of AdKDM3A or KDM3A shRNA ($\times 100$); c-d: KDM3A expression levels were quantified by Western blotting; (n=3). *p< 0.05 vs AdGFP or AdshRNA.

Figure 2

TGFβ1 could induce cardiac fibroblasts conversion, which was accompanied with KDM3A upregulation. a: Western blot showing the expression levels of vimentin, DDR2, CTGF, and α-SMA. b: Representative quantification of Vimentin, DDR2, CTGF, and α-SMA abundance (n=3). c: Western blot showing the expression levels of KDM3A. d: Representative quantification of KDM3A abundance (n=3). *p< 0.05 vs control.

Figure 3

KDM3A regulates the biological function of CFs. a-b: CCK-8 assay showing the proliferation of CFs. c: Representative images of the migration potential of CFs (×200). d: The number of migrated CFs after adenoviral transduction (n=3). *p< 0.05 vs Con-AdGFP or Con-AdshRNA, #p< 0.05 vs TGFβ1-AdGFP or TGFβ1-AdshRNA; ##p< 0.05 vs TGFβ1-AdshKDM3A.

Figure 4

KDM3A is a positive regulator of cardiac fibroblast differentiation, but KDM3A inhibition can reverse myofibroblast conversion through the Smad3-dependent manner. a: Western blots and protein quantification of Collage I, α-SMA, and CTGF (n=3, *P<0.05 vs. Con; #P<0.05 vs. AdshRNA); b: Western blots and protein quantification of Collage I, α-SMA, and CTGF (n=3, *P<0.05 vs. Con; #P<0.05 vs. AdGFP; ##P<0.05 vs. TGFβ1-AdKDM3A). c: Representative western blots and quantitative results of the AdshRNA group and AdshKDM3A group. d: Representative western blots and quantitative results of the AdGFP group and AdKDM3A group. (n=3, *P<0.05 vs. Con; #P<0.05 vs. AdGFP; ##P<0.05 vs. TGFβ1-AdKDM3A). After transfection with AdGFP and AdKDM3A, cardiac fibroblasts were incubated with TGFβ1 for 48 h, while the TGFβ1+AdKDM3A+SIS3 group was incubated with TGFβ1 and 2 μM SIS3-HCL (p-Smad3 inhibitor) for 48 h. The levels of phosphorylated Smad3 and total Smad3 were determined by western blotting.