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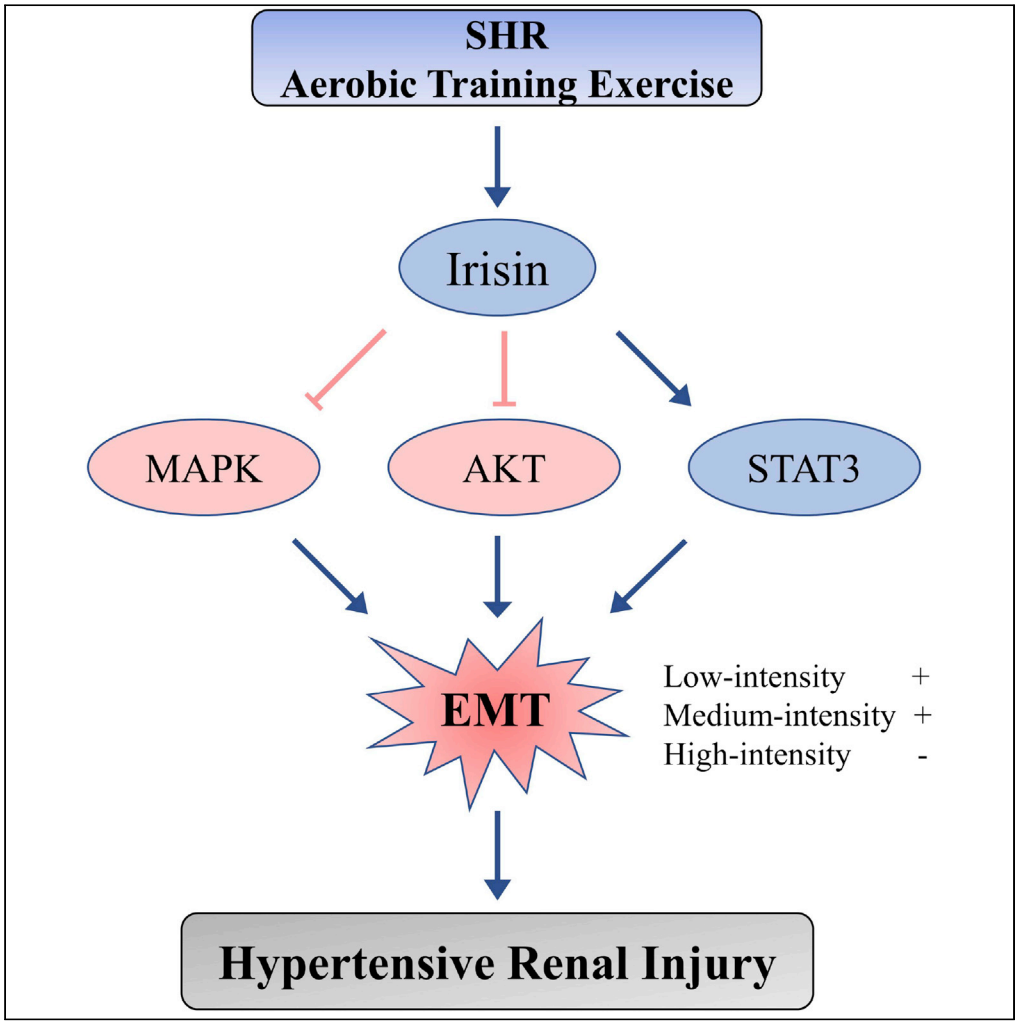

Minghao Luo, Suxin Luo, Yuzhou Xue, ..., Wenyu Dong, Ting Zhang, Shuyuan Cao

shuyuan_cao@hospital.cqmu.edu.cn

Highlights

This study explored the effects of exercise intensity on nephropathy in hypertensive rats

Exercise improved renal function and inhibited EMT, but not at high intensity

The mechanism involved upregulation of irisin and its precursor FNDC5

Different effects of vigorous exercise may be because of irisin activation of STAT3

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Aerobic exercise inhibits renal EMT by promoting irisin expression in SHR

Minghao Luo,[1,2] Suxin Luo,[2] Yuzhou Xue,[2] Qing Chang,[1,3] Hui Yang,[1] Wenyu Dong,[1] Ting Zhang,[4] and Shuyuan Cao[1,4,5,*]

SUMMARY

To determine the effect of aerobic exercise in different intensities on renal injury and epithelial-mesenchymal transformation (EMT) in the kidney of spontaneously hypertensive rats (SHR) and explore possible mechanisms, we subjected SHR to different levels of 14-week aerobic treadmill training. We tested the effects of aerobic exercise on irisin level, renal function, and EMT modulators in the kidney. We also treated angiotensin II-induced HK-2 cells with irisin and tested the changes in EMT levels. The data showed low and moderate aerobic exercise improved renal function and inhibited EMT through promoting irisin expression in SHR. However, high-intensity exercise training had no effect on renal injury and EMT in SHR but did significantly activate STAT3 phosphorylation in the kidney. These results clarify the mechanisms of exercise in improving hypertension-related renal injury and suggest that irisin might be a therapeutic target for patients with kidney injury.

INTRODUCTION

Hypertension, one of the most common chronic diseases, seriously endangers public health.[1] Hypertension-associated damage affects multiple major organs, including the kidney, with hypertensive nephropathy being a main cause of end-stage renal disease (ESRD).[2,3] The basic pathological features of hypertensive nephropathy include inflammation, glomerular sclerosis, tubular atrophy, and progressive interstitial fibrosis.[2] Renal fibrosis is characterized by increased extracellular matrix (ECM) production that results in fibrotic tissue replacing normal kidney tissue and ultimately inducing renal failure.[4–6] Treatments for hypertensive nephropathy remain limited but include attempts to control blood pressure and delay its progression to ESRD.[7]

Several recent studies have shown that exercise has beneficial effects in hypertensive nephropathy or kidney failure, such as lowering blood pressure and alleviating renal fibrosis.[8–14] However, the detailed molecular mechanisms underlying these positive outcomes remain unclear. In experiments on spontaneously hypertensive rats (SHR), aerobic training progressively reduced blood pressure and downregulated the vasoconstrictor axis of the renin-angiotensin system. Regular treadmill training for 8 weeks also partially improved renal fibrosis in SHR via inhibiting the transforming growth factor-β (TGF-β) signaling pathway.[4] Another treadmill experiment in SHR demonstrated that 12 weeks of exercise effectively reduced blood pressure and the renal inflammatory response through downregulating fibrosis pathways.[15]

The epithelial-to-mesenchymal transition (EMT) is a crucial process for organ development and cancer metastasis.[16] The process also plays a key role in the development of renal fibrosis associated with several diseases, including hypertensive nephropathy.[17] During EMT, epithelial cells lose their epithelial features and acquire a mesenchymal phenotype, contributing to renal tubular atrophy and extracellular matrix (ECM) accumulation.[18] Excessive mechanical stimulation or oxidative stress triggers renal tubule interstitial transition, leading to renal tubule fibrosis as renal tubule cells transform into collagen-producing myofibroblasts. Several studies have suggested that canonical mitogen-activated protein kinase (MAPK) signaling is the main mechanism involved in EMT induction, besides, with various pathways linked, including the signal transducer and activator of transcription 3 (STAT3), phosphoinositide 3-kinase/protein kinase B (PI3K/AKT), and wingless/integrated (Wnt) pathways.[17–21] Increasing evidence supports

[1]The Affiliated Rehabilitation Hospital of Chongqing Medical University, Chongqing, China

[2]Department of Cardiology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China

[3]The College of Exercise Medicine, Chongqing Medical University, Chongqing, China

[4]The Fifth Affiliated Hospital of Sun Yat-sen University, Guangdong, China

[5]Lead contact

*Correspondence: shuyuan_cao@hospital.cqmu.edu.cn

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the idea that decreasing EMT inhibits these pathways to alleviate renal fibrosis and protect renal function.[22–25]

Despite these promising findings, few studies have focused on the relationship between exercise training and EMT. In this study, we aimed to determine whether aerobic exercise would suppress EMT and renal injury in hypertensive nephropathy and explored the possible mechanisms.

RESULTS

Effect of exercise training intensity on renal function and EMT of SHR kidney

Measures of renal function (kidney index, Scr, and BUN) were higher in the SHR-S group than in the WKY-S group ($p < 0.05$, Figures 1A–1C). In addition, the kidney index, BUN, and Scr were lower in the SHR-L and SHR-M groups ($p < 0.05$) than in the SHR-S group. However, high-intensity exercise did not improve renal function in SHR ($p > 0.05$).

To examine the effect of aerobic exercise on EMT in SHR, we used immunofluorescence (Figure 1D) and western blotting (Figure 1E) to measure α-SMA, vimentin (mesenchymal phenotype marker), and E-cadherin levels (epithelial cell indicator) in kidney tissue. We also detected the EMT-related transcription factor Snail1 (Figure 1F), which participates in mesenchymal reprogramming and prevents terminal differentiation. The SHR-S group had significantly higher α-SMA, vimentin, and Snail1 expression than the WKY-S, SHR-L, and SHR-M groups ($p < 0.05$). In addition, the SHR-S group had significantly lower E-cadherin levels than the WKY-S, SHR-L, and SHR-M groups. Of interest, SHR-H and SHR-S groups did not differ significantly in α-SMA, vimentin, E-cadherin, and Snail1 levels ($p > 0.05$).

Effect of exercise training intensity on *p*-AKT, *p*-ERK, p-p38, and p-STAT3 in SHR kidney

To determine the effect of exercise training on pathways involved in EMT, we measured renal *p*-AKT, *p*-ERK, p-p38, and p-STAT3 levels with western blotting. The SHR-S group had markedly elevated renal *p*-AKT, *p*-ERK, and p-p38 expression than the WKY-S group ($p < 0.05$, Figure 2A). In addition, the three proteins had significantly lower expression in SHR-L and SHR-M groups than in the SHR-S group ($p < 0.05$). Thus, aerobic exercise attenuated activation of the MAPK and AKT signaling pathways in SHR kidneys. However, SHR kidneys exhibited significantly higher p-STAT3 expression than normal rat kidneys ($p < 0.05$, Figure 2B). Likewise, p-STAT3 levels were significantly higher in the SHR-L, SHR-M, and SHR-H groups than in the SHR-S group ($p < 0.05$). Aerobic exercise had further increased p-STAT3 levels and activated the STAT3 signaling pathway.

Aerobic exercise increased irisin level in SHR

The myokine irisin is secreted by muscles after exercise and has strong positive effects on ameliorating cardiovascular disease and inhibiting renal fibrosis.[26–32] *Fndc5* expression is regulated by PGC-1α.[26] Here, we tested whether aerobic exercise also upregulates the irisin precursor FNDC5 and PGC-1α in rat kidney, and the most commonly used skeletal muscle, gastrocnemius, in the study of myokine.

Compared with the WKY-S group, the SHR-S group had far lower FNDC5 and PGC-1α levels in the kidney and gastrocnemius muscles ($p < 0.05$). The results of ELISA indicated that SHR-S also had significantly lower serum irisin level than WKY-S ($p < 0.05$). In addition, aerobic exercise had a clear influence on irisin and FNDC5. Results from western blotting indicated that FNDC5 expression in SHR-L, SHR-M, and SHR-H kidneys were significantly higher than in SHR-S kidneys ($p < 0.05$; Figures 3A and 3B). Likewise, ELISA showed that the SHR-L, SHR-M, and SHR-H groups had significantly higher irisin levels than the SHR-S group ($p < 0.05$; Figure 3C).

Irisin improved ang II-induced EMT in HK-2 cells

To further verify the effect of irisin on hypertensive renal injury, we incubated HK-2 cells with irisin (1, 10, or 100 ng/mL) in the absence or presence of Ang II. We first confirmed that EMT was induced in response to Ang II (1 μM, 24 h) ($p < 0.05$). Next, we observed that irisin significantly improved Ang II-induced morphological changes in HK-2 cells (Figure 4A). Moreover, at 10 and 100 ng/mL, irisin significantly blocked Ang II-induced increases in α-SMA, vimentin, and Snail1 expression, as well as Ang II-induced decrease in E-cadherin expression ($p < 0.05$; Figures 4B and 4C).

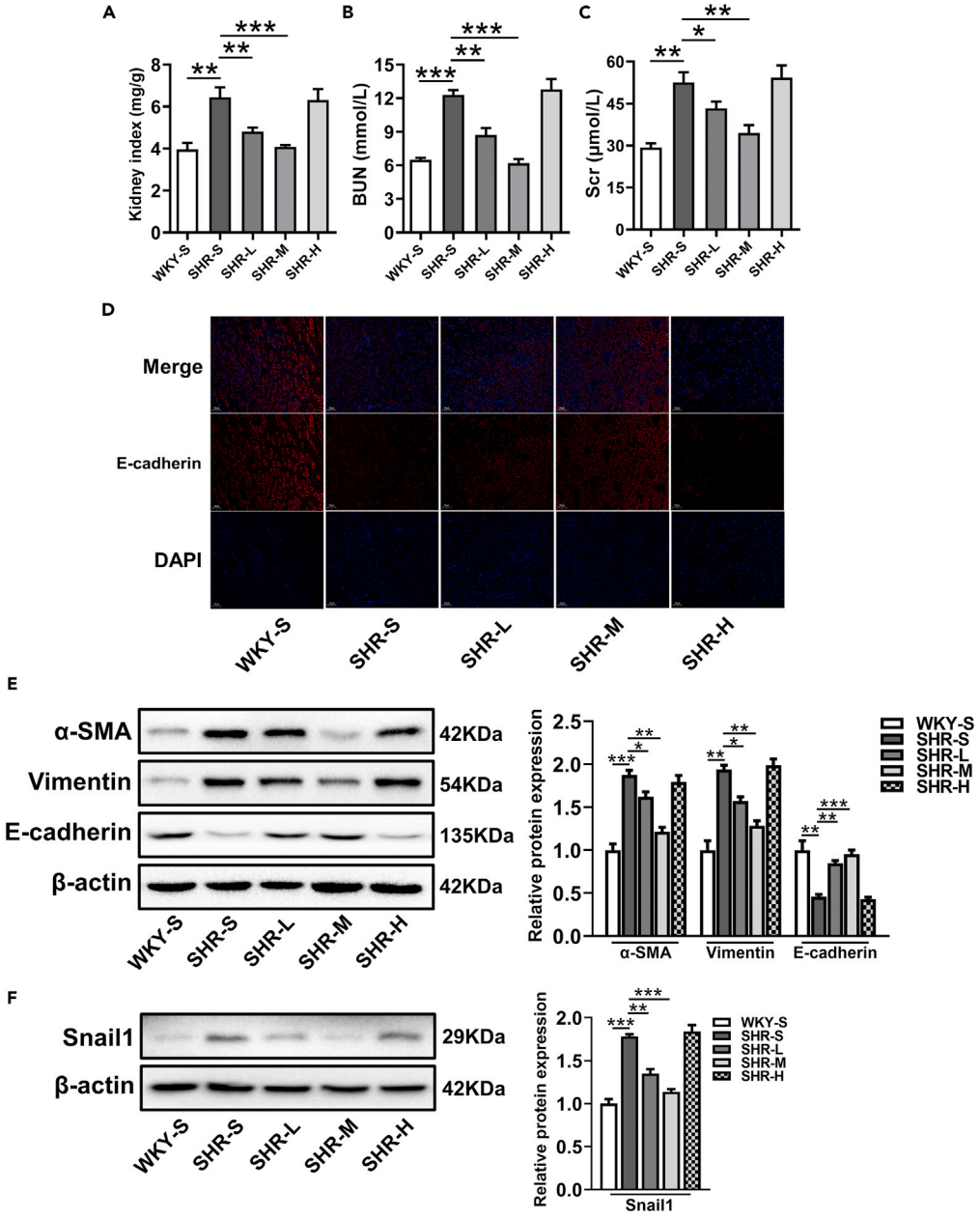

Figure 1. Effect of aerobic exercise at different intensities on renal function and epithelial-mesenchymal transformation (EMT) of the SHR kidney

(A–F) Effects of low-, medium-, and high-intensity aerobic exercise on (A) kidney index, (B) blood urea nitrogen (BUN), (C) serum creatinine (Scr), and EMT of the SHR kidney were tested. The occurrence of EMT was confirmed with immunofluorescence (D) and western blotting (E, F) to measure E-cadherin, α-SMA, vimentin, and Snail1 expression in the kidney. Data are presented as means ± SD n = 8, *p< 0.05, **p< 0.01, ***p< 0.001.

Effect of irisin on ang II-induced changes in *p*-AKT, *p*-ERK, p-p38, and p-STAT3 expression in HK-2 cells

Compared with control cells, Ang II-treated HK-2 cells had increased *p*-AKT, *p*-ERK, and p-p38 expression (p< 0.05, Figure 5A). Irisin treatment at 10 and 100 ng/mL significantly attenuated these Ang II-induced increases in expression levels in HK-2 cells (p< 0.05). Thus, irisin appears to have blocked activation of the MAPK and AKT signaling pathways in Ang II-induced HK-2 cells. However, irisin also increased p-STAT3 levels in Ang II-treated HK-2 cells at all tested concentrations (p< 0.05, Figure 5B).

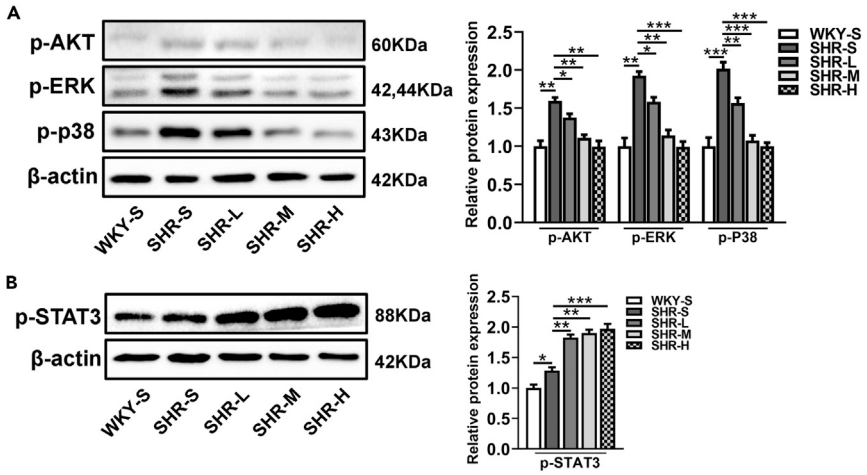

Figure 2. Effect of aerobic exercise at different intensities on MAPK, AKT, and STAT3 signaling pathways of the SHR kidney

(A and B) Western blotting was performed to measure (A) *p*-AKT, *p*-ERK, p-p38, and (B) p-STAT3 levels. Data are presented as means ± SD n = 8, *p< 0.05, **p< 0.01, ***p< 0.001.

Correlation between Stat3 and Fndc5 in CCLE and GTEx databases

The results of our bioinformatics analysis first demonstrated a positive association between *Stat3* and *Fndc5* expression (Pearson's r = 0.12, p< 0.0001) in different cell lines (n = 1017) (Figure 6A). Further exploration the *Stat3* and *Fdnc5* relationship across multiple organs (Figure 6B) revealed that the two genes were most highly correlated in kidney tissue (Pearson's r = 0.81, p< 0.0001). *Stat3* expression was also positively correlated with *Fndc5* transcriptional expression (Pearson's r = 0.24, p< 0.0001).

DISCUSSION

In this study, we investigated whether treadmill exercise at varying intensities (30–40% VO_2max, 45–55% VO_2max, 60–70% VO_2max) influenced renal function and EMT in SHR kidneys and then explored potential

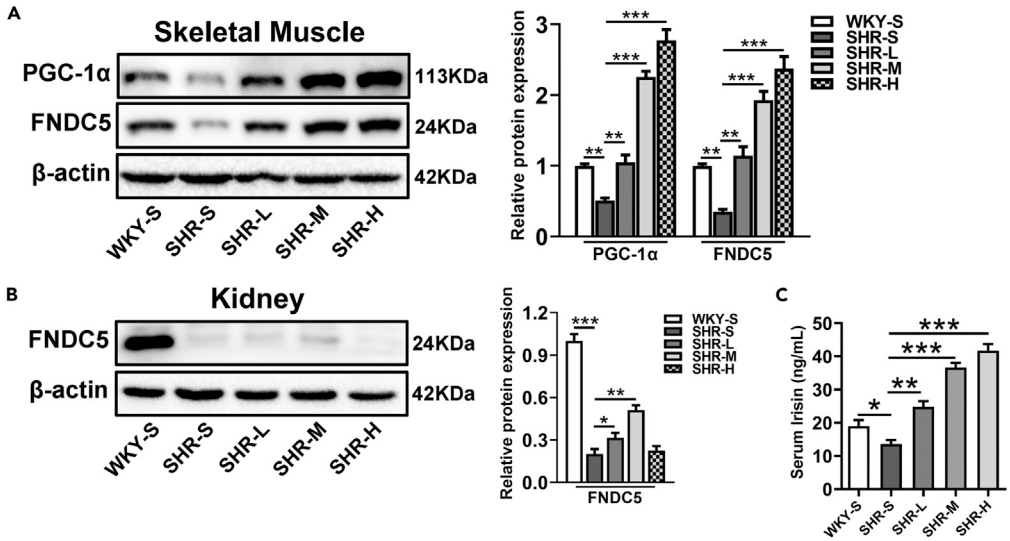

Figure 3. Effect of aerobic exercise at different intensities on irisin level in SHR

(A and B) Western blotting was performed to measure PGC-1α and FNDC5 expression in (A) gastrocnemius and (B) kidney.

(C) ELISA of serum irisin levels. Data are presented as means ± SD n = 8, *p< 0.05, **p< 0.01, ***p< 0.001.

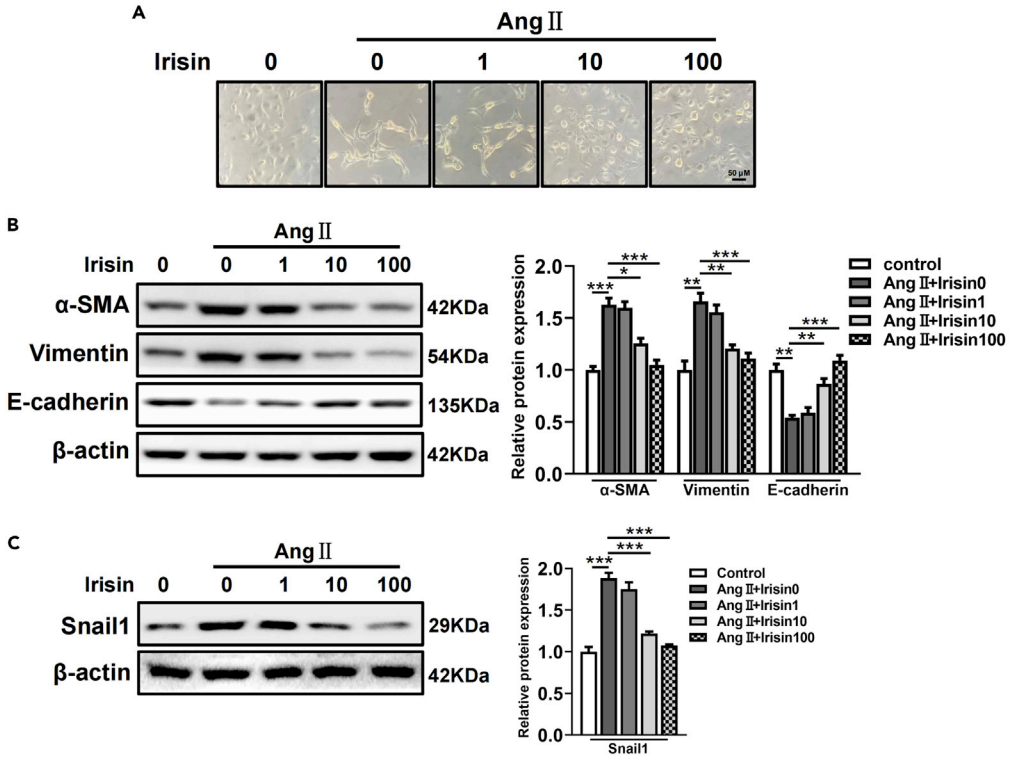

Figure 4. Effect of irisin on Ang II-induced EMT in HK-2 cells

HK-2 cells were incubated with 1, 10, or 100 ng/mL irisin in the absence or presence of Ang II (1 μM).

(A) Changes to morphology.

(B) E-cadherin, α-SMA, and vimentin protein expression.

(C) Snail1 protein expression. Data are presented as means ± SD n = 6, *p< 0.05, **p< 0.01, ***p< 0.001.

mechanisms. We found that low- and medium-intensity, but not high-intensity, training improved renal function and EMT in SHR. In addition, exercise at all three intensities downregulated the activation of MAPK and AKT signaling pathways in SHR kidneys while activating the STAT3 pathway. Aerobic exercise also promoted irisin synthesis and release, which ameliorated Ang II-induced EMT of HK-2 cells *in vitro*. Our results *in vitro* suggested irisin significantly activated the STAT3 pathway; meanwhile, the results of our bioinformatics analysis first demonstrated a positive association between *Stat3* and *Fndc5* expression. These results suggested that the beneficial effects of irisin may be related to the activation of STAT3.

Arterial hypertension is the second cause of ESRD after diabetes mellitus. Kidney dysfunction and hypertension often interact, with nephropathies causing hypertension and high blood pressure damaging the kidney.[1–3] This study is the first to explore how aerobic exercise intensity influences hypertensive-nephropathy-induced EMT. We provided evidence linking the effect of exercise to irisin, a myokine that causes positive outcomes in various diseases. Although classically described as nephroangiosclerosis and hyalinosis of the glomerular tuft, hypertensive nephropathy also affects the interstitium with the development of tubular-interstitial fibrosis (TIF), which ultimately leads to ESRD. Multiple mechanisms can induce TIF, including EMT, an organ-development process that has been observed in several diseases.[7]

Several *in vitro* studies have demonstrated that EMT may be a common mechanism underlying TIF, with the process being driven by factors known to induce hypertension, fibrosis, or both.[5,17] For example, Ang II induces EMT in cell culture models. In this study, we used HK-2 cells to identify EMT as a morphological change from the typical cobblestone pattern of epithelial cells to elongated, spindle-shaped mesenchymal cells. The transition was accompanied by a reduction in the expression level of the epithelial marker E-cadherin with an increase in the expression levels of mesenchymal markers α-SMA and vimentin.[25,33] We recently provided evidence that aged SHR exhibits EMT induction in the kidney through observing the aforementioned changes in epithelial and mesenchymal markers. A common hypothesis is that EMT

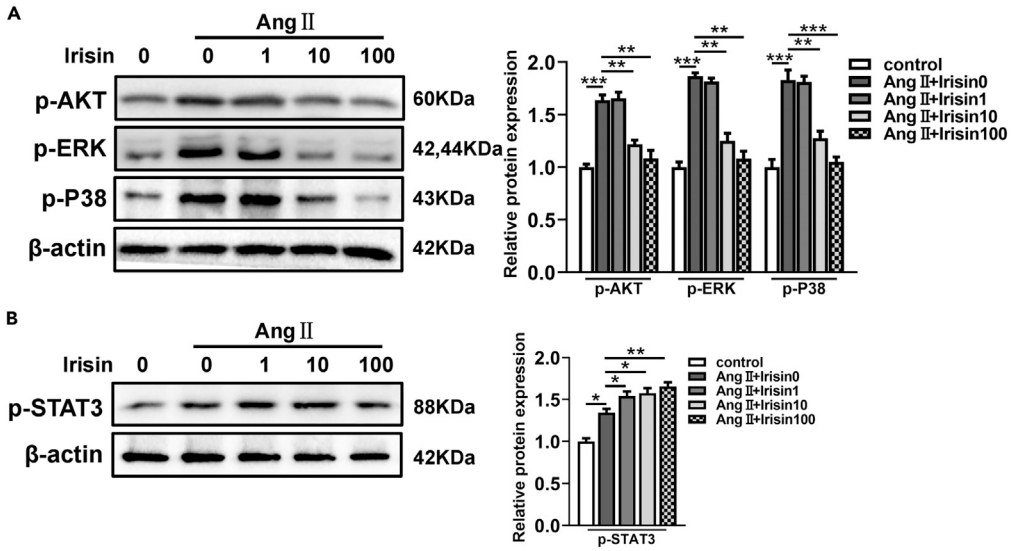

Figure 5. Effects of irisin on Ang II-induced changes to MAPK, AKT, and STAT3 signaling pathways in HK-2 cells

(A and B) HK-2 cells were incubated with 1, 10, or 100 ng/mL irisin in the absence or presence of Ang II (1 μM). Western blotting was used to measured (A) *p*-AKT, *p*-ERK, p-p38, and (B) p-STAT3 levels. Data are presented as means ± SD n = 6, *p< 0.05, **p< 0.01, ***p< 0.001.

allows epithelial cells to escape from a stressful, unfavorable microenvironment. This mesenchymal reprogramming prevents terminal differentiation and appears to be favored when certain transcription factors (e.g., Twist, Zeb1, and Snail1) are overexpressed.[18] Several studies indicate that Snail1 overexpression, for example, causes epithelial plasticity, myofibroblast accumulation, and inflammation. In this study, we observed that moderate aerobic exercise inhibits enhanced Snail1 expression in the SHR kidney.[16,17]

Scheduled exercise in patients with hypertension reduces the risk of cardiovascular death and all-cause death.[12,34–36] Because aerobic exercise is more effective than impedance or stretching exercises for improving symptoms in patients with hypertension, we focused on exploring the effects of varying aerobic training intensity. We obtained maximum aerobic velocity (VO_2max) via measuring exercise speed with a typical progressive exercise test developed for rats. After determining that 40 m/min was 100% of maximum aerobic speed (that is, speed at VO_2max), we formulated low-, medium-, and high-intensity exercise training for SHR.

Previous studies have shown that exercise training inhibits EMT through down-regulating the fibrotic pathway.[22,37,38] Moderate swimming inhibited TGF-β1-induced EMT in mice transplanted with hepatocellular carcinoma cells.[39] Moreover, incremental load training improved renal fibrosis in old mice through regulation of the TGF-β1/TAK1/MMK3/p38MAPK signaling pathway and inducing autophagy activation, which in turn reduced ECM synthesis and delayed EMT.[22] Exercise training also promotes H_2S production while inhibiting the TGF-β1/Smad and LRP-6/β-catenin signaling pathways, EMT, and pulmonary fibrosis.[38] Based on these previous findings, here, we focused on canonical pathways involved in EMT induction, specifically MAPK, AKT, and STAT3 signaling.

A recent study showed that PGC-1α in muscle increases FNDC5 expression and thus circulatory irisin levels.[26] Irisin is generally detectable in animal plasma (including in humans), and its levels are elevated by exercise.[26,28] Irisin upregulation appears to have strong therapeutic potential in cardiovascular disease and related disorders.[29,30] Furthermore, irisin is associated with renal function, proteinuria, and various complications in patients with ESRD, with its presence inhibiting renal fibrosis, alleviating renal injury, and improving renal function.[31,32]

Our results suggest that aerobic exercise significantly increased PGC-1α and FNDC5 levels in skeletal muscle of SHR, as well as increased serum irisin concentration. Notably, high-intensity exercise had little effect on FNDC5 expression in SHR kidneys. Long-term high-intensity training increases lactic acid concentration

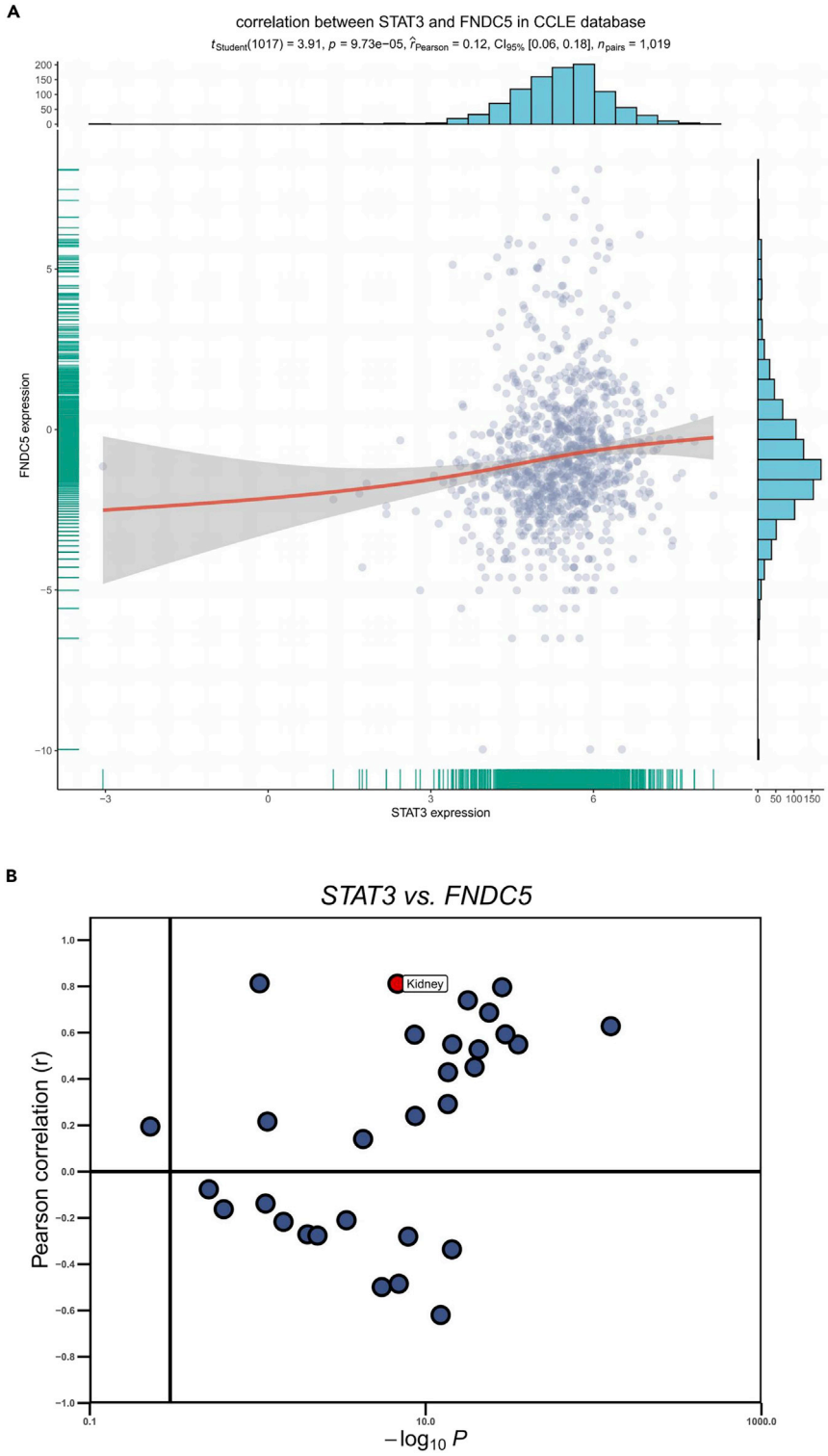

Figure 6. Correlation between *Stat3* and *Fndc5* in CCLE and GTEx databases

(A) Stat3 was positively correlated with Fndc5 expression across different cell lines (n = 1017) in CCLE (Pearson's r = 0.12, p< 0.0001).

(B) After examining data from different organs in GTEx, Stat3 and Fndc5 expression was found to have the highest correlation in kidney tissue (Pearson's r = 0.81, p< 0.0001). Stat3 expression was also positively correlated with Fndc5 transcript levels (Pearson's r = 0.24, p< 0.0001).

through anaerobic metabolism, thus reducing the amount of hydrogen ions (pH < 6.8).[40,41] As lactic acid increases and pH decreases, genes involved in mitochondrial biogenesis (e.g., PGC-1α) are downregulated. In this study, PGC-1α expression increased significantly in the skeletal muscle and decreased in the kidney after high-intensity exercise compared with that after medium- and low-intensity exercise.

Our study agrees with previous findings of improved renal function and renal fibrosis in hypertensive rats after aerobic exercise. Here, medium-intensity training improved renal function more than low-intensity training, mainly reflected in changes to Ang II-induced EMT. However, after a certain level of intensity, aerobic exercise did not improve renal function in hypertensive rats.

The different level of oxidative stress in HICT and MICT on vascular injury in hypertension was also found in our previous study. This is also a possible indication that the results of high-intensity exercise in this study are different from those of other intensities. Training can have positive or negative effects on oxidative stress, depending on intensity, duration, and type. For example, intense exercise causes oxidative stress.[42] Our previous study showed that high-intensity training triggered NO production and could damage vasculature. Similarly, other studies suggest that high-intensity exercise leads to eNOS uncoupling and causes abnormal eNOS function.[42] Thus, high-intensity training may increase iNOS and NO production, with the resultant NO being converted to peroxynitrite (ONOO-), a free radical involved in oxidative stress. We speculated that a threshold may exist between medium- and high-intensity training to explain the differential effect on oxidative stress.

STAT3 is a member of the JAK-STAT pathway that regulates inflammation and fibrosis. STAT3 inhibition reduces type I collagen, fibronectin, vimentin, and α-SMA levels in fibrotic renal cells. Many studies have shown that inhibition of the STAT3 signaling pathway reduces renal inflammatory response and renal interstitial fibrosis.[43–45] Our *in vivo* experiments demonstrated that although high-intensity exercise had no effect on renal EMT, it significantly activated STAT3. *In vitro* experiments then confirmed that irisin upregulates p-STAT3 and activates the STAT3 pathway. This relationship between irisin and STAT3 was also verified with a bioinformatics analysis. Therefore, irisin activation of the STAT3 pathway may be the mechanism that causes high-intensity exercise to have a different effect on hypertensive renal injury than lower intensity exercise. However, the exact mechanism of these effects from exercise remains elusive and is a topic for future research.

In conclusion, the results of this study suggest that exercise exerts distinct effects on renal function in SHR depending on intensity. Low- and medium-intensity exercise significantly ameliorated renal damage and inhibited EMT in SHR through promoting irisin expression. High-intensity training had no effect on renal dysfunction or EMT in SHR, possibly because it activated STAT3.

Limitations of the study

This study did not discuss the mechanisms by which high intensity exercise failed to improve hypertensive nephropathy, but simply demonstrated changes in STAT3 without intervening with STAT3. Besides, this manuscript addresses the effect of aerobic exercise in a spontaneously hypertensive rats context. However, it remains to be tested whether such therapeutic effect is general and extendable to at the clinical level. Whether the exercise prescription in rats is applicable to humans remains to be further verified.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.105990.

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AUTHOR CONTRIBUTIONS

S.C. funded this study; M.L. designed experimental programs and conducted data analysis; S.L. and Q.C. supervised this work and conducted project administration; Y.X. conducted bioinformatics analysis; S.C., H.Y., and W.D. participated in animal experiments; T.Z. participated in data analysis; M.L. wrote and submitted the article.

DECLARATION OF INTERESTS

All authors read and approved the final version of the manuscript, and agreed to the author's order of statements. The authors declare no conflict of interest.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
α-SMA	Proteintech Group	14395-1-AP
Vimentin	Proteintech Group	10366-1-AP
E-cadherin	Proteintech Group	20874-1-AP
Snail1	Proteintech Group	13099-1-AP
PGC-1α	Proteintech Group	66369-1-Ig
HRP-conjugated goat anti-mouse	Proteintech Group	SA00001-1
HRP-conjugated goat anti-rabbit	Proteintech Group	SA00001-2
p-AKT-Ser473	Cell Signaling Technology	#4060
p-ERK-Thr202/Tyr204	Cell Signaling Technology	#4370
p-p38-Thr180/Tyr182	Cell Signaling Technology	#4511
p-STAT3-Tyr705	Cell Signaling Technology	#9145
FNDC5	AiFang	AF301717
β-actin	Bioss	bs-0061R
Fluorescence-conjugated secondary antibody	Beyotime	P0180
Organisms/strains experimental models		
HK-2	ATCC	CRL-2190
SHR rats	Vital River Laboratory	Code:121
WKY rats	Vital River Laboratory	Code:122
Treadmill	SANS Biological Technology	SA101C
Chemicals, peptides, and recombinant proteins		
Irisin	MedChemExpress	HY-P70664
Angiotensin II	MedChemExpress	HY-13948
Critical commercial assays		
Creatinine Assay Kit	Nanjing Jiancheng Bioengineering Institute	C011-2-1
Urea Assay Kit	Nanjing Jiancheng Bioengineering Institute	C013-2-1
Irisin ELISA Kit	Elabscience	E-EL-R2625c
Software and algorithms		
GraphPad Prism 8	GraphPad Software	NA
R 4.0.0	R Core Team, 2020	NA

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact Shuyuan Cao (shuyuan_cao@hospital.cqmu.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

All raw data reported in this paper will be shared by the lead contact upon request. Data S1–S6, original raw data, related to Figures 1, 2, 3, 4, 5, and 6. Data S7, original code related to GTEx. Data S8, original code

related to CCLE. Any additional information required to reanalyze the data reported in this paperis available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals

Thirty-two Eight-week-old healthy male SHR and eight age-matched Wistar-Kyoto (WKY) rats (n = 8) were purchased from the Vital River Laboratory (Beijing, China) and placed under standard pathogen-free conditions with controlled temperature (22 ± 2°C) and 12/12 h light/dark cycle. Subjects were allowed *ad libitum* access to water and food. Animal experiments were carried out in accordance with the National Animal Protection and Use Guidelines and approved by the Animal Ethics Committee of Chongqing Medical University. Rats exercised on a specialized treadmill (SA101C; SANS Biological Technology, Jiangsu, China) connected to software that continuously monitors speed. The treadmill exercise followed protocols described in previous studies.[46–48] Initially, all rats were subjected to an adaptation period comprising 5 days of 60 min running sessions at 8 m/min, between 15:00 and 17:00. Maximal velocity (m/min) and exhaustion time (s) were then determined with a maximum-capacity running test. The actual trials took 14 weeks (2 days of rest per week), with rats exercising at 30–40% (14 m/min), 45–55% (20 m/min), and 60–70% (26 m/min) of maximum exercise capacity for 60 min, also during 15:00–17:00. Treadmill inclination was set to 0.

Rats were separated into three groups for different exercise intensities (n = 8): SHR low-intensity aerobic exercise training (SHR-L), SHR medium-intensity aerobic exercise training (SHR-M), and SHR high-intensity aerobic exercise training (SHR-H). The WKY sedentary (WKY-S) and SHR sedentary (SHR-S) control groups did not participate in training. After exercise training, rats were euthanized to collect tissue and serum, which were stored at −80°C until subsequent analysis.

Cell culture

Human renal proximal tubule epithelial cells (HK-2) were purchased from the American Tissue Culture Collection (VA, USA) and cultured in DMEM (Gibco; Invitrogen, USA) supplemented with 10% FBS and 1% penicillin/streptomycin (Invitrogen, USA) at 37°C with 5% CO_2. To confirm the effect of irisin on renal tubular epithelium *in vitro*, HK-2 cells were incubated for 24 h with 1, 10, or 100 ng/mL of the protein (MedChemExpress, Shanghai, China) in the absence or presence of 1 μM angiotensin II (Ang-II; MedChemExpress). Cell morphology was observed under a light microscope (Leica Microsystems, Germany).

METHOD DETAILS

Western blotting

The chopped kidney and gastrocnemius tissue and cells were washed with ice-cold PBS, and lysed in lysis buffer supplemented with 1% protease and phosphatase inhibitors (Sigma, USA) on ice for 60 minutes. The lysate supernatant was collected after centrifugation at 12,000 rpm for 15minat 4°C. The concentration of the supernatant was determined via Bradford protein assay (Thermo Fisher Scientific, USA). Then mixed with 5X SDS-PAGE sample loading buffer (Beyotime Biotechnology, China) to prepare western blotting protein sample. 40 μg of protein was then used for western blotting.[49–52] Proteins were separated with 10% SDS-PAGE and transferred onto PVDF membranes. After blocking with 5% non-fat milk for 2hat 37°C, membranes were incubated with primary antibodies overnight at 4°C and then with horseradish peroxidase (HRP)-conjugated secondary antibodies at 37°C for 2 h. Bands were detected using chemiluminescence detection reagent (Beyotime, Shanghai, China). Band gray values were analyzed in Image Lab 6.0, and target protein levels were calculated as the ratio of their gray values to the internal control protein (β-actin).

Immunofluorescence staining

Rat kidneys were harvested, frozen in Tissue-Tek OCT media, and sliced sequentially into 10 μm sections using a cryostat. Sections were permeabilized with 0.1% Triton X-100 in phosphate-buffered saline (PBS) for 20 min and then blocked with 5% donkey serum for 1 h and incubated with rabbit anti-E-cadherin (1:100; 20874-1-AP; Proteintech) overnight at 4°C. Next, sections were incubated for 5 min with 4′,6-diamidino-2-phenylindole (DAPI) and then for 2 h with fluorescence-conjugated secondary antibodies (Beyotime, Shanghai, China) in a dark room at room temperature. Slices were visualized under a fluorescence microscope (Leica Microsystems, Germany).

Bioinformatics analysis

Total transcripts per million (TPM) expression profiles were obtained from the Cancer Cell Line Encyclopedia (CCLE) and Genotype-Tissue Expression (GTEx).[53,54] Log-normalized *Stat3* and *Fndc5* expression was qualified through Gencode V19 annotation. *Stat3* and *Fndc5* transcript values were analyzed with Pearson's correlation tests across multiple cell lines and organs using R version 4.0.0 (R Foundation for Statistical Computing). Significance was set at $p < 0.05$ (two-tailed).

QUANTIFICATION AND STATISTICAL ANALYSIS

All experimental data are expressed as means ± standard deviation (SD). Normality of the distribution of data were assessed by Shapiro-Wilk normality test. To calculate the comparisons between 2 groups, normally or nonnormally distributed data were compared using Unpaired 2-tailed Student t tests or Mann-Whitney U tests, respectively. To calculate the comparisons between multiple groups (≥ 3 groups), normally or nonnormally distributed data were compared using one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test or Kruskal-Wallis test followed by the Dunn post hoc test, respectively, in GraphPad version 8.0. Significance was set at $p < 0.05$.