Hepatocyte growth factor inhibits TGF-β1-induced myofibroblast differentiation in tendon fibroblasts: role of AMPK signaling pathway

Qingbo Cui • Songbin Fu • Zhaozhu Li

Received: 24 October 2012 / Accepted: 13 January 2013 / Published online: 1 February 2013
© The Physiological Society of Japan and Springer Japan 2013

Abstract The transforming growth factor-β1 (TGF-β1)-induced myofibroblastic differentiation in tendon fibroblasts was thought to be one of the most important features of scar fibrosis formation, which is associated with occurrence of re-rupture. Previously, we reported that hepatocyte growth factor (HGF) inhibited TGF-β1-induced myofibroblast differentiation and extracellular matrix deposition in the Achilles tendon of rats. Here, we investigated the potential molecular mechanisms underlying the inhibitory effect of HGF on TGF-β1-induced myofibroblastic differentiation. We found that treatment with HGF (10, 20, and 40 ng/ml) increased phosphorylation of adenosine monophosphate kinase (AMPK) and acetyl-CoA carboxylase (ACC) in tendon fibroblasts. Pharmacological inhibition of the AMPK signaling pathway using compound C, a specific blocker of AMPK signaling, remarkably attenuated the inhibitory effect of HGF on TGF-β1-induced myofibroblastic differentiation in tendon fibroblasts. Moreover, small interfering RNA (siRNA)-mediated knockdown of AMPKα1 subunit decreased the inhibitory effect of HGF on TGF-β1-induced myofibroblastic differentiation in tendon fibroblasts. Finally, overexpression of constitutively active AMPKα1, which led to constitutive activation of the AMPK signaling pathway in tendon fibroblasts, mimicked the inhibitory effect of HGF on the TGF-β1-induced myofibroblastic differentiation. Our study therefore suggests that HGF inhibits TGF-β1-induced myofibroblastic differentiation via an AMPK signaling pathway-dependent manner in tendon fibroblasts.

Keywords HGF • TGF-β1 • Myofibroblastic differentiation • AMPK

Introduction

Fibroblasts are active participants in creating connective tissue and are instrumental in wound healing owing to their exceptional ability to undergo various interconversions between related but distinctly different cell types. In the 1970s, the transient appearance and disappearance of “myofibroblasts” in the granulation tissue of healing cutaneous wounds was described [1]. Myofibroblasts are cells that are in between a fibroblast and a smooth muscle cell in terms of differentiation and have been defined by their ability to express contractile proteins, including α-smooth muscle actin (α-SMA), collagen, type I, alpha 1 (Col I-α1), collagen, type III (Col III), fibronectin, etc. [2]. The responsive profile of transforming growth factor β1 (TGF-β1) in healing tissue is thought to play a critical role in the healing of tendons and ligaments. It induces proliferation of connective tissue cells [3, 4]. Importantly, TGF-β1 stimulates matrix contraction, cell growth, and collagen production in fibroblast and then produces tissue-like fibroplasia, a fibronectin matrix-dependent event [5, 6]. TGF-β receptor type II (TGF-βRII) is found to be responsive and essential for TGF-β’s function [7].

Tendon or ligament injury, a common problem caused by physical and recreational activities, often results in scar fibrosis formation, characterized by myofibroblast differentiation and overproduction of extracellular matrix (ECM) [8, 9]. After scar fibrosis formation, the biochemical and
mechanical properties of the healed tendon were greatly decreased. For example, after the first incidence of Achilles tendon rupture, the re-rupture rate can be as high as approximately 20 % [10]. TGF-β1 was found to be the major inducer of scar tissue formation in tendon fibroblasts [11] by activating myofibroblastic differentiation. Thus, to blunt or disrupt the TGF-β1-induced myofibroblastic differentiation in tendon fibroblasts may be a promising therapeutic target for tendon injury.

Recently, cytokines such as hepatocyte growth factor (HGF) were reported to attenuate TGF-β1-induced myofibroblastic differentiation. HGF is a heterodimer molecule composed of a large 69-kDa subunit and a small 34-kDa subunit [12], and is a humoral mediator of liver regeneration [13]. HGF prevents the initiation and progression of chronic renal fibrosis in a wide variety of animal models [14]. HGF suppresses type III collagen expression in human renal fibroblasts [15] and in rat ligament fibroblasts [16]. Our group previously demonstrated that HGF inhibits TGF-β1-induced myofibroblastic differentiation and ECM deposition in the Achilles tendon of rats [17]. However, how HGF inhibits TGF-β1-induced myofibroblastic differentiation is largely unknown. In this study, we explored the role of the AMPK signaling pathway in the inhibitory effect of HGF on TGF-β1-induced myofibroblastic differentiation.

Materials and methods

Animals

Adult male Sprague–Dawley rats weighing 250–260 g were supplied by the Beijing Vital River Laboratory Animal, Inc. Rats were housed in a facility with controlled temperature (23 ± 2 °C) and lighting (0800–2000 hours), with free access to tap water and food. All procedures were performed in compliance with and approved by the Animal Care and Usage Committee at Harbin Medical University.

Isolation and culture of primary tendon fibroblasts

The tendon fibroblasts were isolated and cultured as described previously [17]. Briefly, the Achilles tendons of Sprague–Dawley rats were harvested to culture tendon fibroblasts. The tendons were isolated and washed in sterile saline containing 1 % penicillin/streptomycin and cut into 1-mm sections. The sections were subjected to collagenase type II (0.1 %) digestion overnight in DMEM (Sigma, St. Louis, MO, USA) containing 5 % FBS, penicillin/streptomycin, and amphotericin. Then, samples were placed in an orbital shaker at 37 °C [18]. The solution was spun for 10 min at 3000×g. The supernatant was removed and the pellet was washed two times with the above medium. The isolated cells and remaining tissue were plated in a 75-mm² tissue culture flask and placed in an incubator (5 % CO₂ and 37 °C) [19]. All experiments were performed using the cells at passages 4–6.

Experiments and groups

Tendon fibroblasts were grown to approximately 70 % confluence, and switched to serum-starved medium (0.1 % FBS) for 8 h before further treatments. Then, the cells were treated with TGF-β1 (5 ng/ml, Peprotech, Rocky Hill, NJ) or TGF-β1 plus different concentrations of HGF (10, 20, and 40 ng/ml, Peprotech, Rocky Hill, NJ). For the following mechanism study, the AMPK inhibitor compound C (Sigma-Aldrich) was also added into cell culture wells to block the AMPK signaling pathway [20].

siRNA-mediated RNA interference

We knocked down AMPK using siRNAs targeting AMPK-α1 (sc-270142, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) in tendon fibroblasts with Lipofectamine-LTX with Plus Reagent (Invitrogen, Carlsbad, CA) [21]. The transfection was performed according to the manufacturer’s instructions. Western blotting was used to confirm the efficiency of knockdown.

Transfection of constitutively active AMPK-α1 subunit

We overexpressed plasmid of constitutively active AMPK-α1 (caAMPK-α1, addgene, plasmid no. 27632) in tendon fibroblasts using Lipofectamine-LTX with Plus Reagent (Invitrogen, Carlsbad, CA). Western blotting analysis with specific primary antibody against the N-terminal epitope of both exogenous AMPK and caAMPKα2 was performed to confirm the constitutive activation of AMPK signaling in transfected cells. At 3 days post-transfection, the cells were treated with TGF-β1.

RNA extraction and quantitative real-time PCR analysis

Quantitative real-time PCR analysis was performed as previously described [22, 23]. Total RNA was extracted from cell tissues using Trizol, and 1 μg RNA was reverse transcribed to cDNA. Quantitative PCR was performed using Opticon Monitor 3 real-time PCR system (Bio-Rad) and SYBR Premix Ex Taq Mixture (Takara) with specific primers. The PCR reactions were initiated with denaturation at 95 °C for 10 s, followed by amplification with 40 cycles at 95 °C for 10 s, and annealing at 60 °C for 20 s.
(two-step method). Finally, melting curve analysis from 60 to 85 °C was performed [24]. Data were evaluated with Opticon Monitor 3 software [25]. The sequences of the primers were as follows: α-SMA, CATAACCTAAAG CCAACA (sense), CAGTGATAGG (anti-sense); Col I, CACCAGACGCCAGG (sense), GCAAAGTTTCCAGAAGC (anti-sense); Col III, CTACACCTGCTCCTATGCA (sense), GCCACCACGACT (anti-sense); Fibronectin, ACTCGCTCCTGGCATCAG (sense), TCCTGGCTCGTCCGT (anti-sense); β-actin, ATCTGCGTCTGGCTAGCC (sense), CCGCTGCTGCGATACT (anti-sense). All data were normalized to β-actin expression (2^(-ΔΔCt) methods) [26].

**Immunoblotting**

Immunoblotting analyses were performed as described previously [26, 27]. Cell samples were subjected to 10 % SDS-PAGE, and transferred onto PVDF membranes at 100 V for 1–2 h. After being blocked in blocking buffer with 5 % (w/v) nonfat milk and 0.1 % (v/v) Tween 20 in phosphate-buffered saline for 4 h, the membrane was incubated with specific primary antibody (phospho-AMPK, total-AMPK, phospho-acetyl-CoA carboxylase [ACC] and total-ACC; Cell Signaling Technology, Beverly, MA) and then followed by HRP-labeled secondary antibody [28]. The membranes were then detected using the enhanced chemiluminescence system as described previously [29].

**Statistical analysis**

Data are expressed as mean ± SD. Differences were evaluated by two-tailed Student’s t test or ANOVA. Statistical significance was set at P < 0.05.

**Results**

**HGF activates AMPK signaling pathway in tendon fibroblasts**

As shown in Fig. 1a, treatment with different concentrations of HGF (10, 20, and 40 ng/ml) induced phosphorylation of AMPK in tendon fibroblasts. We also measured the phosphorylation of ACC, a critical downstream factor of AMPK. Accordingly, phosphorylation of ACC was increased significantly by HGF in tendon fibroblasts (Fig. 1b). Because there was no difference in the increase of AMPK phosphorylation between the three concentrations, the moderate concentration of HGF (20 ng/ml) was used in the following experiments.

**Administration of AMPK inhibitor compound C abolishes the inhibitory effect of HGF on the TGF-β1-induced myofibroblastic differentiation in tendon fibroblasts**

Previously, we showed that HGF attenuated TGF-β1-induced fibrogenic responses in the tendon in vivo [17]. In this investigation, we studied this phenotype in tendon fibroblasts. Before the incubation of TGF-β1, the tendon fibroblasts were flat. At 72 h post TGF-β1 treatment, some tube-like structures were observed (Fig. 2a). As shown in Fig. 2b–e, administration of TGF-β1 (10 ng/ml) for 48 and 72 h induced significant upregulation in mRNA levels of several markers for myofibroblastic differentiation (α-SMA, Col I α1, Col III, and fibronectin), suggesting that TGF-β1 induced myofibroblastic differentiation in tendon fibroblasts. In agreement with previous in vivo data [17], supplementation with HGF obviously inhibited the changes of mRNA levels of these markers (Fig. 2b–e). Notably,
treatment with compound C, a well-established AMPK signaling pathway inhibitor, greatly attenuated the inhibitory effect of HGF on TGF-β1-induced effects (Fig. 2b–e). These results indicate that HGF may decrease TGF-β1-induced myofibroblastic differentiation via the AMPK signaling pathway partly.

Blocking AMPK signaling by knocking down of AMPKα1 subunit disrupts the inhibitory effect of HGF on the TGF-β1-induced myofibroblastic differentiation in tendon fibroblasts

Considering that the chemical agent (compound C) may have potential unspecific effects, we used a more specific method to block the AMPK signaling pathway. Knocking down of AMPKα1 using AMPKα1-targeting siRNA significantly decreased the AMPKα1 subunit protein level (>70%, data not shown). As shown in Fig. 3a, HGF treatment stopped the TGF-β1-induced myofibroblastic differentiation in tendon fibroblasts in wild-type (WT) and siRNA-scramble transfected cells but not in siRNA-AMPKα1 transfected cells (Fig. 3a). The AMPKα1 knockdown disrupted the inhibitory effect of HGF on the TGF-β1-induced myofibroblastic differentiation in tendon fibroblasts (Fig. 3b–e).

Constitutive activation of AMPK signaling mimics the inhibitory effect of HGF on the TGF-β1-induced myofibroblastic differentiation in tendon fibroblasts

Next, we further studied whether constitutive activation of AMPK signaling can mimic the inhibitory effect of HGF on the TGF-β1-induced myofibroblastic differentiation in tendon fibroblasts. We overexpressed the caAMPKα1 plasmid (Fig. 4a) into tendon fibroblasts, which induced constitutive activation of AMPK signaling (Fig. 4b). Interestingly, compared with WT and vector-control transfected cells, the caAMPKα1-transfected cells showed blunted TGF-β1-induced myofibroblastic differentiation (Fig. 5a). The increases of mRNA levels α-SMA, Col I α1, Col III, and fibronectin (Fig. 5b–e), indicating constitutive activation of AMPK signaling, mimics the inhibitory effect of HGF on the TGF-β1-induced myofibroblastic differentiation in tendon fibroblasts.

Discussion

In the present study, using an in vitro cell model, we found that HGF inhibited TGF-β1-induced myofibroblastic differentiation via an AMPK signaling pathway-dependent manner in tendon fibroblasts. First, HGF activated the
AMPK signaling pathway in tendon fibroblasts. Second, either pharmacological or genetic inactivation of the AMPK signaling pathway greatly revoked the inhibitory effect of HGF on TGF-β1-induced myofibroblastic differentiation. Finally, constitutive activation of AMPK signaling using caAMPKα1 overexpression in tendon fibroblasts mimicked the inhibitory effect of HGF on the TGF-β1-induced myofibroblastic differentiation.

HGF has a remarkable ability to block myofibroblast transition. Yang and Liu [30] demonstrated that HGF abrogated the α-SMA expression and E-cadherin depression triggered by TGF-β1 in renal tubular epithelial cells in a dose-dependent manner. Moreover, injections of exogenous HGF blocked myofibroblast activation and drastically prevented renal interstitial fibrosis in the obstructed kidneys [30]. HGF also blocked the IL-1α-induced myofibroblastic
differentiation of renal tubular epithelial cells and inhibited the secretion of fibronectin from these cells [31]. In addition, HGF contributed to the inhibition of vitamin D on renal interstitial myofibroblast activation [32]. Besides renal cells, HGF was found to inhibit TGF-induced myofibroblastic differentiation in human retinal pigment epithelial cells [33], primary vocal fold fibroblasts [34], hepatic stellate cells [35], and human cardiac fibroblasts [36]. Our group also demonstrated the anti-fibrotic effect of HGF in the Achilles tendon [17]. Therefore, the inhibitory effect of HGF on fibrosis is well documented. However, the underlying mechanisms have not been fully elucidated. Kwiecinski et al. [35] provided an explanation for this inhibitory effect: upregulation of miRNA-29 by HGF takes part in the anti-fibrogenic effects of HGF. Apart from this, there was no other study on the detailed molecular mechanisms of HGF in TGF-β1-induced myofibroblastic differentiation.

AMPK is a heterotrimeric protein that is highly conserved from yeast to humans and functions as a key sensor of fuel and energy status [37]. The stimulation of the AMPK signaling pathway by AMP or nutrition shortage results in the repression of many anabolic processes (such as protein, fatty acid, and cholesterol synthesis) and activation of several catabolic processes (such as glucose/fatty acid uptake and oxidation) [37]. Thus, AMPK plays an important role in the adaptive response of cells to nutrient deprivation in vitro and to exercise- or ischemia-induced energy stresses in vivo. Recently, there were two reports on the relationship between myofibroblast differentiation and the AMPK signaling pathway. Cai et al. [38] found that adiponectin, an adipokine, inhibited lipopolysaccharide-induced adventitial fibroblast migration and transition to myofibroblasts via an AMPK-dependent manner. Conversely, Cieslik et al. [39] showed that 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR), a chemical AMPK activator, induced TGF-β-dependent development of myofibroblasts but did not affect adipogenesis in cardiac fibroblasts from aged mice. In our study, we observed that HGF activated the AMPK signaling pathway. We also demonstrated that treatment with the chemical AMPK inhibitor compound C or knocking down of the AMPKα1 subunit using siRNA-mediated RNA interference abolished the inhibitory effect of HGF on the TGF-β1-induced myofibroblastic differentiation in tendon fibroblasts. Moreover,
overexpression of the constitutive activation of AMPKα1 mimicked the inhibitory effect of HGF on the TGF-β1-induced myofibroblastic differentiation in tendon fibroblasts. Our data supported the notion that activation of the AMPK signaling pathway by HGF inhibited but did not promote TGF-β1-induced myofibroblastic differentiation in tendon fibroblasts.

In summary, we demonstrated a critical role of AMPK in the inhibitory effect of HGF on TGF-β1-induced myofibroblastic differentiation in tendon fibroblasts, which may provide a promising therapeutic strategy for the treatment of fibrosis during tendon/ligament healing.

Acknowledgments
This study was supported by the National Scientific Foundation of China (81101349), the National Scientific Foundation of China (81110024), the General Financial Grant from the China Postdoctoral Science Foundation (2011M500684), the General Financial Grant from the Heilongjiang Postdoctoral Science Foundation (LBJ-Z11072), the Opening Project of the Key Laboratory of Medical Genetics (Harbin Medical University), Heilongjiang Higher Education Institutions and the Science Research Foundation of the Department of Science and Technology of Harbin (2011RFQYS101).

Conflict of interest
All the authors declared no conflict of interests.

References
1. Gabbiani G, Hirschel BJ, Ryan GB, Statkov PR, Majno G (1972) Granulation tissue as a contractile organ. A study of structure and function. J Exp Med 135:719–734
2. Gabbiani G (2003) The myofibroblast in wound healing and fibrocontractive diseases. J Pathol 200:500–503
3. Haase HR, Clarkson RW, Waters MJ, Bartold PM (1997) Growth factor modulation of mitogenic responses and proteoglycan synthesis by human periodontal fibroblasts. J Cell Physiol 174:353–361
4. Hamilton JA, Butler DM, Stanton H (1994) Cytokine interactions promoting DNA synthesis in human synovial fibroblasts. J Rheumatol 21:797–803
5. Clark RA, McCoy GA, Folkvord JM, McPherson JM (1997) TGF-β1 stimulates cultured human fibroblasts to proliferate and produce tissue-like fibroplasia: a fibronectin matrix-dependent event. J Cell Physiol 170:69–80
6. Tuan TL, Song A, Chang S, Younai S, Nimmi ME (1996) In vitro fibroplasia: matrix contraction, cell growth, and collagen production of fibroblasts cultured in fibrin gels. Exp Cell Res 223:127–134
7. Zhao Y, Young SL (1996) Requirement of transforming growth factor-β (TGF-β) type II receptor for TGF-β-induced proliferation and growth inhibition. J Biol Chem 271:2369–2372
8. Wang ED (1998) Tendon repair. J Hand Ther 11:105–110
9. Wight TN, Potter-Perigo S (2011) The extracellular matrix: an active or passive player in fibrosis? Am J Physiol Gastrointest Liver Physiol 301:G950–G955
10. Lynch RM (2004) Achilles tendon rupture: surgical versus nonsurgical treatment. Accid Emerg Nurs 12:149–158
11. Campbell BH, Agarwal C, Wang JH (2004) TGF-β1, TGF-β3, and PGE(2) regulate contraction of human patellar tendon fibroblasts. Biomech Model Mechanobiol 2:239–245
12. Nakamura T, Teramoto H, Ichihara A (1986) Purification and characterization of a growth factor from rat platelets for mature parenchymal hepatocytes in primary cultures. Proc Natl Acad Sci U S A 83:6489–6493
13. Nakamura T, Nishizawa T, Hagiya M, Seki T, Shimoni M, Sugimura A et al (1989) Molecular cloning and expression of human hepatocyte growth factor. Nature 342:440–443
14. Liu Y (2004) Hepatocyte growth factor in kidney fibrosis: therapeutic potential and mechanisms of action. Am J Physiol Renal Physiol 287:F7–16
15. Mou S, Wang Q, Shi B, Gu L, Ni Z (2009) Hepatocyte growth factor suppresses transforming growth factor-beta-1 and type III collagen in human primary renal fibroblasts. Kaohsiung J Med Sci 25:577–587
16. Jiang D, Jiang Z, Han F, Zhang Y, Li Z (2008) HGF suppresses the production of collagen type III and α-SMA induced by TGF-β1 in healing fibroblasts. Eur J Appl Physiol 103:489–493
17. Cui Q, Wang Z, Jiang D, Qu L, Guo J, Li Z (2011) HGF inhibits TGF-β1-induced myofibroblast differentiation and ECM deposition via MMP-2 in Achilles tendon in rat. Eur J Appl Physiol 111:1457–1463
18. Ezaki J, Matsumoto N, Takeda-Ezaki M, Komatsu M, Takahashi K, Hiraoka Y et al (2011) Liver autophagy contributes to the maintenance of blood glucose and amino acid levels. Autophagy 7:727–736
19. Waltz P, Carchman EH, Young AC, Rao J, Rosengart MR, Kaczorowski D et al (2011) Lipopolysaccharide induces autophagic signaling in macrophages via a TLR4, heme oxygenase-1 dependent pathway. Autophagy 7:315–320
20. Jiang K, Song KS, Shin S, Kim N, Jeong S, Oh HR et al (2011) Docosahexaenoic acid induces autophagy through p53/AMPK/mTOR signaling and promotes apoptosis in human cancer cells harboring wild-type p53. Autophagy 7:1348–1358
21. Malik SA, Shen S, Marino G, BenYounes A, Mauri MC, Kroemer G (2011) BH3 mimetics reveal the network properties of autophagy-regulatory signaling cascades. Autophagy 7:914–916
22. Wang P, Xu TY, Guan YF, Su DF, Fan GR, Miao CY (2009) Perivascular adipose tissue-derived visfatin is a vascular smooth muscle cell growth factor: role of nicotinamide mononucleotide. Cardiovasc Res 81:370–380
23. Wang P, Guan YF, Du H, Zhai QW, Su DF, Miao CY (2012) Induction of autophagy contributes to the neuroprotection of nicotinamide phosphoribosyltransferase in cerebral ischemia. Autophagy 8:77–87
24. Loffler AS, Alers S, Dieterle AM, Keppeler H, Franz-Wachtel M, Kauczor HU et al (2011) BH3 mimetics reveal the network properties of Beclin 1-independent autophagy in cerebral ischemia. Autophagy 7:727–736
25. Malik SA, Shen S, Marino G, BenYounes A, Mauri MC, Kroemer G (2011) BH3 mimetics reveal the network properties of autophagy-regulatory signaling cascades. Autophagy 7:914–916
26. Grishchuk Y, Ginet V, Truttmann AC, Clarke PG, Puyal J (2011) BH3 mimetics reveal the network properties of Beclin 1-independent autophagy in cerebral ischemia. Autophagy 7:727–736
27. Wang P, Xu TY, Guan YF, Su DF, Fan GR, Miao CY (2009) Perivascular adipose tissue-derived visfatin is a vascular smooth muscle cell growth factor: role of nicotinamide mononucleotide. Cardiovasc Res 81:370–380
28. Wang P, Guan YF, Du H, Zhai QW, Su DF, Miao CY (2012) Induction of autophagy contributes to the neuroprotection of nicotinamide phosphoribosyltransferase in cerebral ischemia. Autophagy 8:77–87
29. Loffler AS, Alers S, Dieterle AM, Keppeler H, Franz-Wachtel M, Kauczor HU et al (2011) BH3 mimetics reveal the network properties of Beclin 1-independent autophagy in cerebral ischemia. Autophagy 7:727–736
30. Waltz P, Carchman EH, Young AC, Rao J, Rosengart MR, Kaczorowski D et al (2011) Lipopolysaccharide induces autophagic signaling in macrophages via a TLR4, heme oxygenase-1 dependent pathway. Autophagy 7:315–320
31. Wang P, Guan YF, Du H, Zhai QW, Su DF, Miao CY (2012) Induction of autophagy contributes to the neuroprotection of nicotinamide phosphoribosyltransferase in cerebral ischemia. Autophagy 8:77–87
32. Loffler AS, Alers S, Dieterle AM, Keppeler H, Franz-Wachtel M, Kauczor HU et al (2011) BH3 mimetics reveal the network properties of Beclin 1-independent autophagy in cerebral ischemia. Autophagy 7:727–736
33. Wang P, Tian WW, Song J, Guan YF, Miao CY (2011) Deficiency of NG2(+) cells contributes to the susceptibility of stroke-prone spontaneously hypertensive rats. CNS Neurosci Ther 17:327–332
34. Dong LW, Hou YJ, Tan YX, Tang L, Pan YF, Wang M et al (2011) Prognostic significance of Beclin 1 in intrahepatic cholangiocellular carcinoma. Autophagy 7:1222–1229
35. Wang P, Xu TY, Guan YF, Tian WW, Viollet B, Rui YC et al (2011) Nicotinamide phosphoribosyltransferase protects against ischemic stroke through SIRT1-dependent adenosine monophosphate-activated kinase pathway. Ann Neurol 69:360–374
36. Grishchuk Y, Ginet V, Truttmann AC, Clarke PG, Puyal J (2011) Beclin 1-independent autophagy contributes to apoptosis in cortical neurons. Autophagy 7:1115–1131
37. Wang P, Zhang RY, Song J, Guan YF, Xu TY, Du H et al (2012) Loss of AMP-activated protein kinase-alpha2 impairs the insulin-sensitizing effect of calorie restriction in skeletal muscle. Diabetes 61:1051–1061
30. Yang J, Liu Y (2002) Blockage of tubular epithelial to myofibroblast transition by hepatocyte growth factor prevents renal interstitial fibrosis. J Am Soc Nephrol 13:96–107
31. Liu XY, Luo H, Wang M, Chen HZ, Su BH, Li Z et al (2005) Effects of hepatocyte growth factor on IL-1alpha triggered tubular epithelial-myofibroblast transdifferentiation and fibronectin secretion in vitro. Sichuan Da Xue Xue Bao Yi Xue Ban 36:9–12
32. Li Y, Spataro BC, Yang J, Dai C, Liu Y (2005) 1,25-Dihydroxyvitamin D inhibits renal interstitial myofibroblast activation by inducing hepatocyte growth factor expression. Kidney Int 68:1500–1510
33. Gamulescu MA, Chen Y, He S, Spee C, Jin M, Ryan SJ et al (2006) Transforming growth factor beta2-induced myofibroblastic differentiation of human retinal pigment epithelial cells: regulation by extracellular matrix proteins and hepatocyte growth factor. Exp Eye Res 83:212–222
34. Vyas B, Ishikawa K, Duflo S, Chen X, Thibeault SL (2010) Inhibitory effects of hepatocyte growth factor and interleukin-6 on transforming growth factor-beta1 mediated vocal fold fibroblast-myofibroblast differentiation. Ann Otol Rhinol Laryngol 119:350–357
35. Kwiecinski M, Noetel A, Efimova N, Trebicka J, Schievenbusch S, Strack I et al (2011) Hepatocyte growth factor (HGF) inhibits collagen I and IV synthesis in hepatic stellate cells by miRNA-29 induction. PLoS ONE 6:e24568
36. Okayama K, Azuma J, Dosaka N, Iekushi K, Sanada F, Kusunoki H et al (2012) Hepatocyte growth factor reduces cardiac fibrosis by inhibiting endothelial-mesenchymal transition. Hypertension 59:958–965
37. Lage R, Dieguez C, Vidal-Puig A, Lopez M (2008) AMPK: a metabolic gauge regulating whole-body energy homeostasis. Trends Mol Med 14:539–549
38. Cai XJ, Chen L, Li L, Feng M, Li X, Zhang K et al (2010) Adiponectin inhibits lipopolysaccharide-induced adventitial fibroblast migration and transition to myofibroblasts via AdipoR1-AMPK-iNOS pathway. Mol Endocrinol 24:218–228
39. Cieslik KA, Trial J, Entman ML (2011) Defective myofibroblast formation from mesenchymal stem cells in the aging murine heart rescue by activation of the AMPK pathway. Am J Pathol 179:1792–1806