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The pathway to muscle fibrosis depends on myostatin stimulating the differentiation of fibro/adipogenic progenitor cells in chronic kidney disease

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Abstract

Fibrosis in skeletal muscle develops after injury or in response to chronic kidney disease (CKD) but the origin of cells becoming fibrous tissue and the initiating and sustaining mechanisms causing muscle fibrosis are unclear. We have identified muscle fibro/adipogenic progenitor cells (FAPs) that potentially differentiate into adipose tissues or fibrosis. We also demonstrated that CKD stimulates myostatin production in muscle. Therefore, we tested whether CKD induces myostatin which stimulates fibrotic differentiation of FAPs leading to fibrosis in skeletal muscles. We isolated FAPs from mouse muscles and found that myostatin stimulates their proliferation and conversion into fibrocytes. *In vivo*, FAPs isolated from EGFP-transgenic mice (FAPs-EGFP) were transplanted into muscles of mice with CKD or into mouse muscles that were treated with myostatin. CKD or myostatin stimulated FAPs-EGFP proliferation in muscle and increased α-smooth muscle actin expression in FAP-EGFP cells. When myostatin was inhibited with a neutralizing peptibody (a chimeric peptide-Fc fusion protein), the FAP proliferation and muscle fibrosis induced by CKD were both suppressed. Knocking down Smad3 in cultured FAPs interrupted their conversion into fibrocytes indicating that myostatin directly converts FAPs into fibrocytes. Thus, counteracting myostatin may be a strategy for preventing the development of fibrosis in skeletal muscles of patients with CKD.

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Disclosures

None

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Keywords

Fibrosis; Chronic kidney disease (CKD); Mesenchymal progenitor cells; Myostatin

Introduction

Diseases that injure the kidney, heart, liver, lung, skin and potentially other organs can initiate a series of complex cellular and molecular events producing fibrosis which leads to cellular dysfunction and ultimately, organ failure [1;2]. Despite developments in understanding the pathogenesis of fibrosis in many organs, there are no routinely successful treatments that block the development of fibrosis. The difficulties in designing anti-fibrotic treatments are related in part to problems with identifying the origin of cells that produce the proteins which contribute to the development of fibrosis and uncovering the pathways that lead to fibrosis [2]. For example, LeBleu et al., investigated cells that develop into fibrocytes in damaged kidneys and concluded that different cells contribute to the development of fibrosis in the injured kidneys [3]. There also is evidence that cells initiating fibrosis in injured kidney or lung tissues arise following epithelial-mesenchymal transition (EMT) [4;5]. Others report that kidney fibrosis develops following activation of bone marrow-derived cells or perivascular fibrocytes although the involvement of these cells in elaboration of collagen type 1 is controversial [3;4;6]. Responses to injury in other organs such as the liver involve precursors of perivascular mesenchymal cells (e.g., hepatic stellate cells). They contribute to the development of hepatic fibrosis but the involvement of other cells has not been excluded [7]. In the generation of cardiac fibrosis, there is evidence that TGF-β1 initiates EMT with increased activity of fibrocytes although fibrosis in the heart also could involve other cells [8].

Mechanisms that initiate fibrosis in skeletal muscles has received minimal attention and is not even discussed in a popular review [2]. In investigating muscle fibrosis, we found that changes in IGF-1 in mice with chronic kidney disease (CKD) contributes to fibrosis in injured muscles but we did not identify specific cells that produce fibrosis nor did the pathway that stimulates muscle fibrosis [1]. In injured skeletal muscles, one potential precursor of fibrosis is the satellite cell because they possess multilineage capabilities including myogenic, adipogenic and fibrogenic properties [9;10]. In fact, impaired functions of satellite cells in mice with CKD or in aging mice is associated with skeletal muscle fibrosis [1;11]. Mice bearing satellite cell mutations (e.g., in mice with *Pax7* knockout (KO) or with genetic deletions of *Myogenin, Myf5* or *MyoD)* experience accumulation of adipocytes and muscle fibrosis after muscle injury [12–15]. However, an evaluation of lineage-tracing in MyoD-Cre/R26R-EYFP mice concluded that satellite cells do not spontaneously develop into adipocytes or fibrocytes [16]. Besides satellite cells, we and others have uncovered a mesenchymal progenitor cells also called fibro/adipogenic progenitor cells (FAPs) in muscle that can develop into adipocytes or fibrocytes [17–20]. These FAPs express PDGFRα and contribute to adipocyte formation in skeletal muscles of mice that are treated with glucocorticoids (GC) [17]. FAPs also can stimulate myogenesis [17;20]. Although mice engineered to overexpress PDGFRα develop systemic fibrosis, the pathway stimulating fibrosis following muscle injury has not been identified [21]. In these experiments, we used *in*

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vitro and *in vivo* techniques to identify how CKD stimulates the development of fibrosis in skeletal muscles. Our results reveal that CKD-induced fibrosis in skeletal muscles originates from FAPs and blocking myostatin suppresses muscle fibrosis in mice with CKD.

Results

CKD induces fibrosis in skeletal muscle

CKD in male mice was created by subtotal nephrectomy in 2 stages over 2 weeks. Results from CKD mice were compared to those of sham-operated, pair fed mice [22]. After recovery from surgery, mice were fed 40% protein yielding BUN values >80 mg/dL vs. ~20 mg/dL in control mice [22;23]. After 5 months, Sirius Red staining of tibialis anterior (TA) muscles revealed increased collagen deposition in CKD mice vs. control mice (Figure 1A). In gastrocnemius muscles, CKD led to higher mRNAs of fibrosis markers (Figure 1B). Thus, CKD stimulates fibrosis in muscles.

To evaluate whether CKD also stimulates muscle fibrosis in a model of muscle injury, we injected cardiotoxin into mouse TA muscles, a standard model of muscle injury [24]. After 14 days of muscle injury, mice with CKD exhibited a significant increase ($P<0.05$) in collagen deposition (Figure 1C). Muscle injury in mice with CKD also had a significant increase in the mRNAs of fibrotic genes (Figure 1D). Thus, CKD in mice increases muscle fibrosis in both uninjured (Figure 1A,B) and injured muscles (Figure 1C,D).

Myostatin converts FAPs into fibrocytes

We confirmed our finding that myostatin expression is increased in muscles of mice with CKD (Supplemental Figure S1 A,B) [22]. We hypothesized that the increase in myostatin production could stimulate muscle fibrosis and examined isolated FAPs to determine if they can be converted into adipocytes or fibrocytes[17–19]. FAPs were isolated from muscles of mice using FACS (Fluorescence-activated Cell Sorting) as described (Figure 2A) [17;25]. We treated FAPs with 100 nM recombinant myostatin and found there was an increase in their proliferation rate plus increased expression of cyclin D1 and the proliferating cell nuclear antigen (PCNA) (Figure 2 B,C). Thus, myostatin stimulates FAP proliferation *in vitro,* associated with increases in p-Smad2 and p-Smad3 proteins (Figure 2C).

Previously, we found that FAPs were capable of differentiating into adipocytes when they were cultured in an adipogenic differentiation media containing dexmethasone (Dex) [17]. Since Dex stimulates myostatin expression in muscle, we examined whether myostatin, like Dex could raise adipogenesis from FAPs [26;27]. We added 40 nM myostatin to the adipogenic differentiation media which lacked Dex, there was suppression of adipocyte formation from FAPs vs. results obtained in media containing Dex (Figure 3A). Instead, the added myostatin induced most of the FAPs to develop into α-SMA-positive cells, characteristic of fibrosis (Figure 3B). The protein expression pattern of adipogenic and fibrotic genes were consistent with the immunostaining results: four days after FAPs were exposed to adipogenic media plus Dex, the proteins of adipogenic genes in FAPs were increased. But, when FAPs were cultured in media plus myostatin instead of Dex, protein levels of fibrotic genes were increased (Figure 3C). Differentiation of FAPs into adipocytes or fibrocytes led to a decrease

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in the expression of the mesenchymal progenitor marker, PDGFRα (Figure 3C). The mRNAs of adipogenic and fibrotic markers were also consistent with the changes in expressed proteins following 2 to 4 days treatment (Figure 3D). We conclude that myostatin stimulates fibrotic differentiation of FAPs resulting in increases in markers of fibrosis.

In mice with CKD, muscle fibrosis develops from FAPs

To identify whether the muscle fibrosis found in CKD arises from FAPs, we examined an acute stimulus of fibrosis, muscle injury. Firstly, the number of FAPs ($P<0.05$) in injured muscles of mice with CKD was greater than results in injured muscles of control mice. There also is a slight increase in FAPs in uninjured muscle of CKD (Figure 4A). To examine whether this increase in FAPs was due to FAP proliferation, we co-immunostained sections of injured TA muscles with antibodies against Ki67 and the FAP marker, PDGFRα. In injured muscles of mice with CKD, the doubly labelled cells were significantly increased ($P<0.05$), indicating that CKD stimulates the proliferation of FAPs in injured muscles (Figure 4B).

To determine if CKD also induces FAPs to differentiate into fibrosis *in vivo*, we isolated FAPs from transgenic mice that ubiquitously express EGFP. These FAPs were transplanted into TA muscles of 4 groups of mice to determine if transplanted FAPs-EGFP can be stimulated to express α-SMA: 1) control mice (CTRL); 2) control mice with injured TA muscles; 3) mice with CKD; and 4) mice with CKD and injured TA muscles. The transplanted FAPs did express α-SMA in mice of groups 2, 3 and 4; the largest increase in α-SMA expression was in mice with muscle injury and CKD (group 4; Figure 4C). CKD or injury also stimulated FAP cell proliferation since we found more $EGFP^+$ cells in muscles of mice in group 2, 3 or 4 with vs. control mice even though the same number of FAPs was transplanted into muscles of mice.

To determine if myostatin can mediate the differentiation of FAPs into fibrosis, we transplanted freshly isolated FAPs from EGFP mice into TA muscles. This was followed by an injection of myostatin-soaked beads into the same areas of TA muscles. With this technique, we confined the influence of myostatin from the beads to the same area of muscle bearing FAPs transplanted from EGFP mice. Ten days later, injection of beads bearing myostatin into muscles transplanted with FAPs increased the expression of a-SMA in FAPs that were labelled with EGFP. This pattern of stimulating a-SMA occurred in non-injured and injured muscles of mice that were injected with myostatin (Figure 4D). We conclude that myostatin is stimulated by CKD to activate both the proliferation and differentiation of FAPs into fibrocytes in muscle.

CKD stimulates myostatin to induce FAP differentiation into fibrous tissue

To examine if myostatin mediates muscle fibrosis in mice with CKD, we blocked responses to myostatin by injecting mice with a neutralizing, anti-myostatin peptibody every other day [22]. After 5 days, TA muscles were injured by injecting cardiotoxin while the peptibody treatment was continued. Four groups of mice were examined: 1) control mice (CTRL) treated with PBS; 2) control mice treated with the peptibody; 3) CKD mice were treated

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with PBS; and 4) CKD mice were treated with the peptibody. We also injured right TA muscle of 4 groups of mice to speed up the fibrosis.

After 3 days of TA muscle injury in CKD mice treated peptibody (Group 4), there was a significant ($P<0.05$) decrease in the number of FAPs vs. CKD mice treated with PBS (Group 3; Figure 5A). After 14 days of muscle injury, peptibody treatment sharply reduced Sirius Red staining of muscles (Group 4 vs. Group 3 mice) (Figure 5B). Treatment also decreased the mRNAs of α-SMA and collagen-1 ~50% (Figure 5C; $P<0.05$, group 4 vs. group 3). In Group 2, control mice, myostatin inhibition with the peptibody reduced mRNA levels of fibrosis markers but the differences were not statistically different from results in Group 1) (Figure 5C). The treatment also promoted regeneration of muscles in mice with CKD (Supplemental Figure S2). We conclude that myostatin expression is necessary to stimulate CKD-induced muscle fibrosis.

Myostatin stimulates fibrotic differentiation of FAPs directly

To examine if myostatin directly stimulates differentiation of FAPs into fibrous tissue, in cultured FAPs, we knocked down Smad3 by transfecting FAPs with a lentivirus that expresses Smad3 SiRNA; lentivirus expressing scrambled SiRNA served as a control. After 48 h, the FAPs were treated with myostatin and cells were co-immunostained with anti-Smad3 and α-SMA. Knock down Smad3 blocked myostatin-induced α-SMA expression in FAPs (Figure 6A). We also found that knock down Smad3 does not interfere with the expression of PPARγ indicating that Smad3 is not responsible for adipogenic differentiation of FAPs (Figure 6B). Smad3 knock down did, however, suppress myostatin-induced expression fibrotic markers indicating that myostatin directly stimulates FAPs to differentiate into fibrocytes via a Smad3 signaling pathway (Figure 6B).

Discussion

Fibrosis in skeletal muscles develops in several chronic illnesses including CKD and muscular dystrophies [2;28;29]. In these disorders, myofibers are replaced by extracellular matrix, impairing skeletal muscle functions and counteracting cell and gene therapies for muscle disorders [28]. Unfortunately, there are no routinely inhibitory treatments that reverse muscle fibrosis, in part, because the identities of cells producing fibrosis are controversial and pathways initiating muscle fibrosis are poorly defined. Using three strategies, we have investigated the process by which fibrotic tissue develops in skeletal muscle: we studied mice with CKD-induced muscle fibrosis; we investigated whether mesenchymal progenitor cells (FAPs) in muscle transform into fibrocytes; and we evaluated whether myostatin signaling stimulates FAP differentiation into muscle fibrosis. We found that CKD stimulates myostatin production and converts FAPs into muscle fibrocytes via a Smad3 pathway. Thus, we have identified how CKD stimulates fibrosis in muscle.

In CKD patients, myostatin production in muscle is inversely proportional to the patient's eGFR [30;31]. In mice with CKD, myostatin levels are increased and linked to the development to muscle atrophy which is suppressed by inhibiting myostatin [22]. How does CKD stimulate myostatin production? One possibility is that CKD activates inflammation, signified by increases in circulating cytokines; when we inhibited myostatin in mice with CKD, muscle

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cytokine expression was suppressed [1;22]. Another link between inflammation and myostatin is that mice fed a high-fat diet have increases in circulating TNF-α and IL-6 levels which are reduced in myostatin KO mice [32]. Moreover, there is a NF-κB consensus sequence in the myostatin promoter and its activation raises myostatin expression [22;33]. We and others find that myostatin stimulates muscle production of IL-6 via MEK and p38 MAPK resulting in impaired satellite cell activity, reduced Akt phosphorylation and causing muscle atrophy [1;22;34]. Finally, we find that in CKD, Stat3 activation stimulating myostatin expression through the transcription factor, C/EBPδ [23]. Myostatin-induced, catabolic pathways in muscles of mice with CKD are summarized in Figure 7.

The origins of cells developing into muscle fibrosis are controversial. Primary myoblasts or C2C12-derived myoblasts can become muscle fibroblasts [35;36]. Other cells developing into fibroblasts include resident or circulating bone marrow [29]. We find that FAPs are not only major precursors of muscle fibrocytes but also have multipotential properties including the ability to stimulate satellite cells or their conversion into osteoblasts or adipocytes [17;20]. We find (Supplemental figure S3 A) that CKD also increases ($P<0.05$) the mRNAs of adipocyte genes, PPARγ and C/EBPα in muscle suggesting that CKD-induces adipogenesis from FAPs. This is possible because the increase in glucocorticoids in CKD or diabetes is known to stimulate conversion of FAPs into adipocytes [17;37;38]. We speculate that patients with diabetic CKD have increased ectopic accumulation of adipose tissue causing insulin resistance [17].

Besides myostatin, other factors may also affect FAP differentiation. For example, we found that loss of one IGF-1 receptor allele in mice responding to muscle injury actually increases muscle fibrosis [1]. In mice with CKD and intact IGF-1 receptor genes, similar results were obtained (Supplemental Figure S3 A,B) raising the possibility that impaired IGF-1 signaling is a mediator of both fibrosis and adipocyte infiltration in muscles of mice with CKD. These results suggest future studies examining whether there is a synergetic relationship between myostatin and IGF-1 signaling that regulates FAP differentiation. There also are interest and contraversal results in the expression of Gdf11 as a means of reversing age-related cardiac hypertrophy with improvement in muscle function [39–42]. The role of this factor in CKD is uncertain, because muscle-specific deletion of the Gdf11 gene in mice with $Mstn^{+/+}$ or $Mstn^{-/-}$ backgrounds, did not increase muscle mass, fiber number, or fiber type [43].

What novel findings have we uncovered? Firstly, CKD can stimulate FAP conversion into fibrocytes (Figure 1). Secondly, myostatin mediates the development of muscle fibrosis (Figure 2–4) [22]. Thirdly, myostatin inhibition suppresses muscle fibrosis and increases muscle mass in mice with CKD (Supplemental Figures 2, Figure 5) [22]. Fourthly, myostatin directly stimulates the conversion of FAPs into fibroblasts via Smad3 signaling but it does not stimulate the transformation of FAPs into adipocytes (Figure 6) [44]. Our results suggest that inhibition of myostatin may be a therapeutic strategy for depressing the development of muscle fibrosis in patients with CKD or chronic, progressive myopathies. Our results may be clinically relevant because Uezumi et al., noted that PDGFRα is present in human muscles while Arrighi et al., concluded that functional properties in human muscles and adipocytes are indistinguishable from events in mice [45;46].

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Concise Methods

Animals

Animal experimental procedures were approved by the Baylor College of Medicine IACUC Committee. Anesthetized, C57/BL6 male mice, 8 to 10 week old underwent subtotal nephrectomy in two stages [22]; mice with a BUN >80 mg/dl were studied. Sham-operated control mice were pair fed with CKD mice so control mice ate the same amount of food as the CKD mouse on the prior day. Tibialis anterior (TA) muscles were injured by a standard technique of injecting cardiotoxin (Sigma-Aldrich, St. Louis, MO); contralateral muscles were injected with PBS [1;24]. To inhibit myostatin, CKD and control mice were subcutaneously injected with an anti-myostatin peptibody (5 mg/kg) every other day [22]. This neutralizing, anti-myostatin peptibody suppresses myostatin rather than activin A activity and has an IC50 ~ 1.2 nM (Atara Biopharmaceuticals, Westlake Village, CA).

FAP transplantation

FAPs (PDGFRα positive cells negative for CD31, CD45 and integrin-α7) were isolated using FACS and the Baylor Cell Sorting Core Facility [17;25]. Freshly isolated FAPs (5×10^4) were resuspended in 30 μl Matrigel (BD Bioscience, San Jose, CA) and transplanted into TA muscles of mice with CKD or mice muscle treated with myostatin. To confine myostatin to a specific area in muscle, heparin-agarose beads (125–250 μm diameter; Sigma Aldrich) were incubated in 100 μg/ml myostatin for 1 h at RT. Myostatin-soaked beads were mixed with freshly isolated FAPs and injected into TA muscles. After 10 days, muscles were removed from euthanized mice and used for histological analysis or frozen in liquid nitrogen and stored at −80°C until proteins or RNAs were evaluated.

Cell culture

Isolated FAPs were plated in Matrigel-coated (BD Bioscience) tissue culture plates and cultured in growth media (GM: high-glucose DMEM-supplemented media with 2.5 ng ml^{-1} bFGF (Invitrogen), 20% FBS and 10% heat-inactivated horse serum). Growth media was then switched to adipogenic differentiation media (DMEM with 10% FBS, 11.5 μg/ml isobutylmethylxanthine (IBMX) and 1 μg/ml insulin) plus 1 μM dexamethasone (Dex) or 40 nM recombinant myostatin. The proliferation index was calculated as Ki67-positive cells divided by the number of cells in a defined area. Investigators counting Ki67-positive cells were masked as to treatments.

Histology and imaging

PBS was perfused through the left ventricle of anesthetized mice and tissues were removed and processed for cryo-sectioning followed by staining with H&E or Sirius Red. Immunostaining was performed using antibodies against perilipin, PDGFRα, α-SMA, GFP or Ki67 as described [23].

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RT-PCR analysis

RT-PCR was performed as described with relative gene expression calculated from cycle threshold (Ct) values using GAPDH or 18S as an internal control (relative expression = $2^{(\text{sample Ct} - \text{GAPDH Ct})}$) [47]. Primers sequences are provided upon request.

Statistical Analysis

Results are expressed as means ±SEM. Significance testing was performed using one-way ANOVA followed by pair-wise comparisons using the Student-Newman-Keuls test. Statistical significance was set at P<0.05. A minimum of three replicates were performed for each experimental condition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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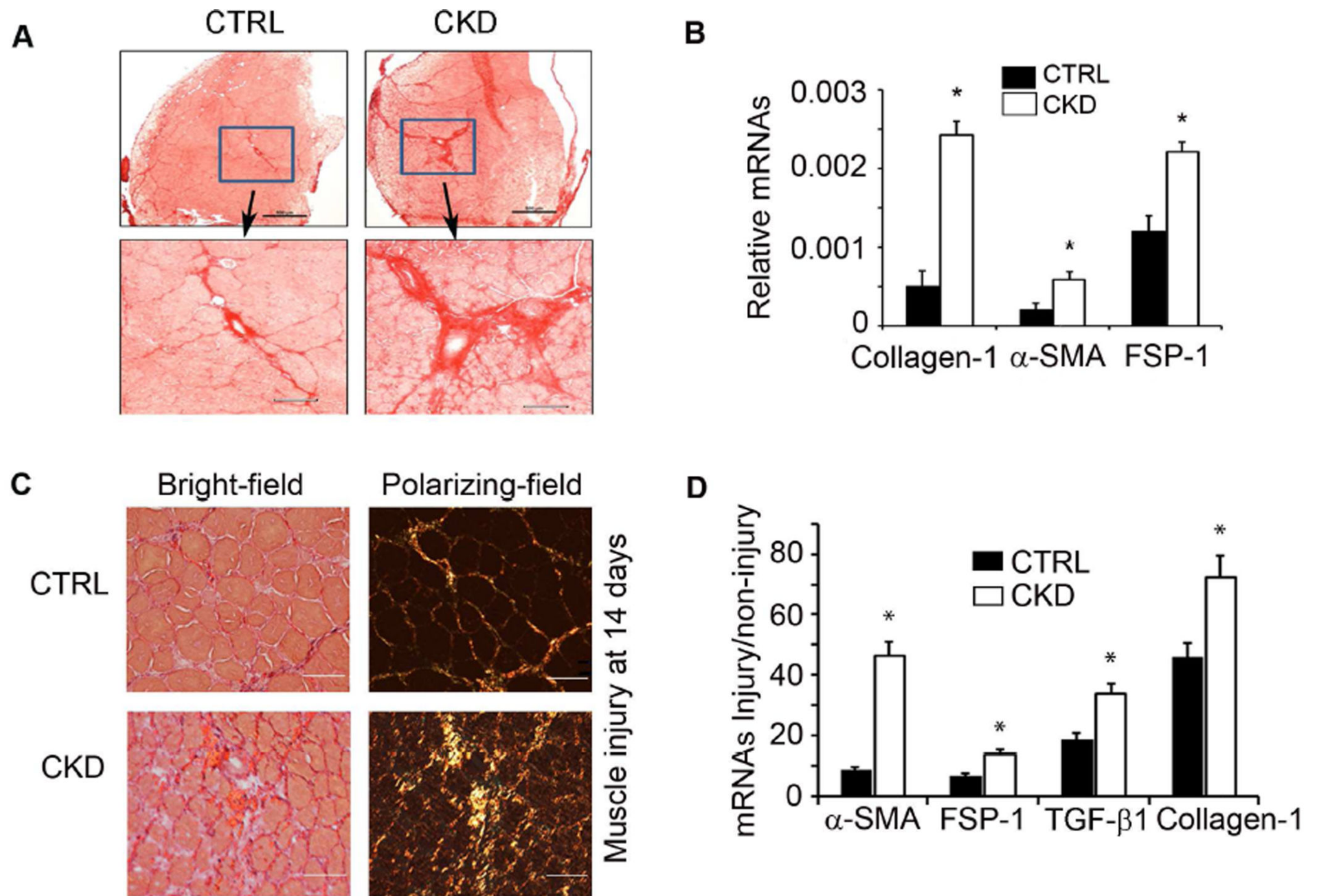

Figure 1. CKD increases muscle fibrosis

A. Cryo-cross sections of TA muscles were subjected to Sirius Red stainning (bar=50μm); CKD was associated with increased collagen deposition. B. Fibrotic marker mRNAs measured by RT-PCR were higher in gastrocnemius muscles of mice with CKD (n=4; *, $P<0.05$ vs. control). C. TA muscles were injured by cardiotoxin injection and compared to the contralateral muscle injected with PBS. After 14 days, Sirius red stained cryo-cross-sections of muscles of CKD mice (bar=50μm) revealed more fibrous tissue. D. mRNAs of markers of fibrosis genes were increased in injured muscles of CKD mice (n=4; *, $P<0.05$ vs. control).

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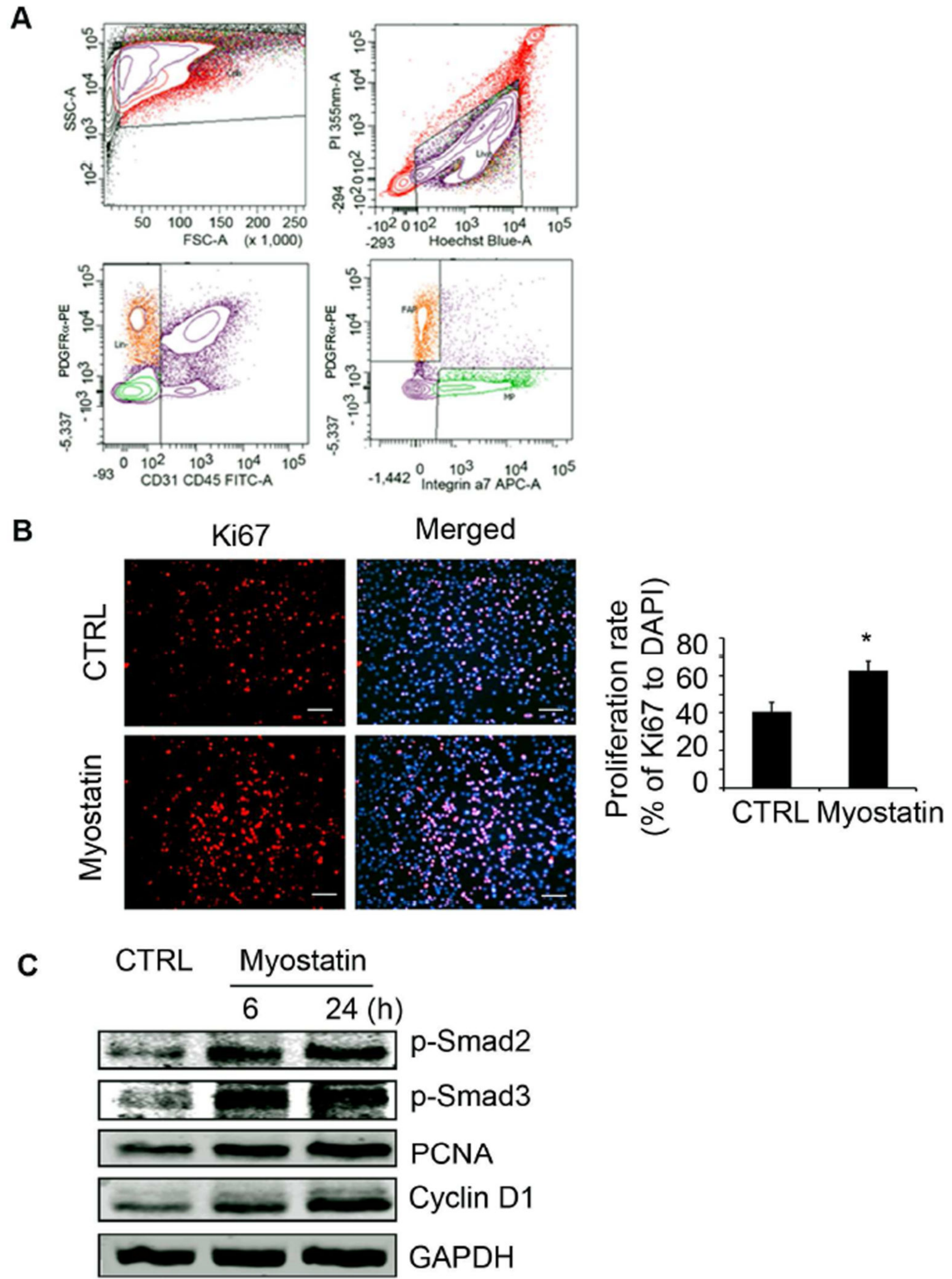

Figure 2. Myostatin induces FAP proliferation

A. FACS-isolated FAPs were positive for PDGFRα but negative for CD31, CD45 and integrinα7 (orange cell area). B. FAPs treated with myostatin for 24h were immunostained with anti-Ki67 and its proliferation index is increased (n=3; *, P<0.05 vs. no myostatin). C. FAPs treated with myostatin for 6 or 24h had increased levels of p-Smad2/3 and PCNA and Cyclin D1 (n=3 repeats).

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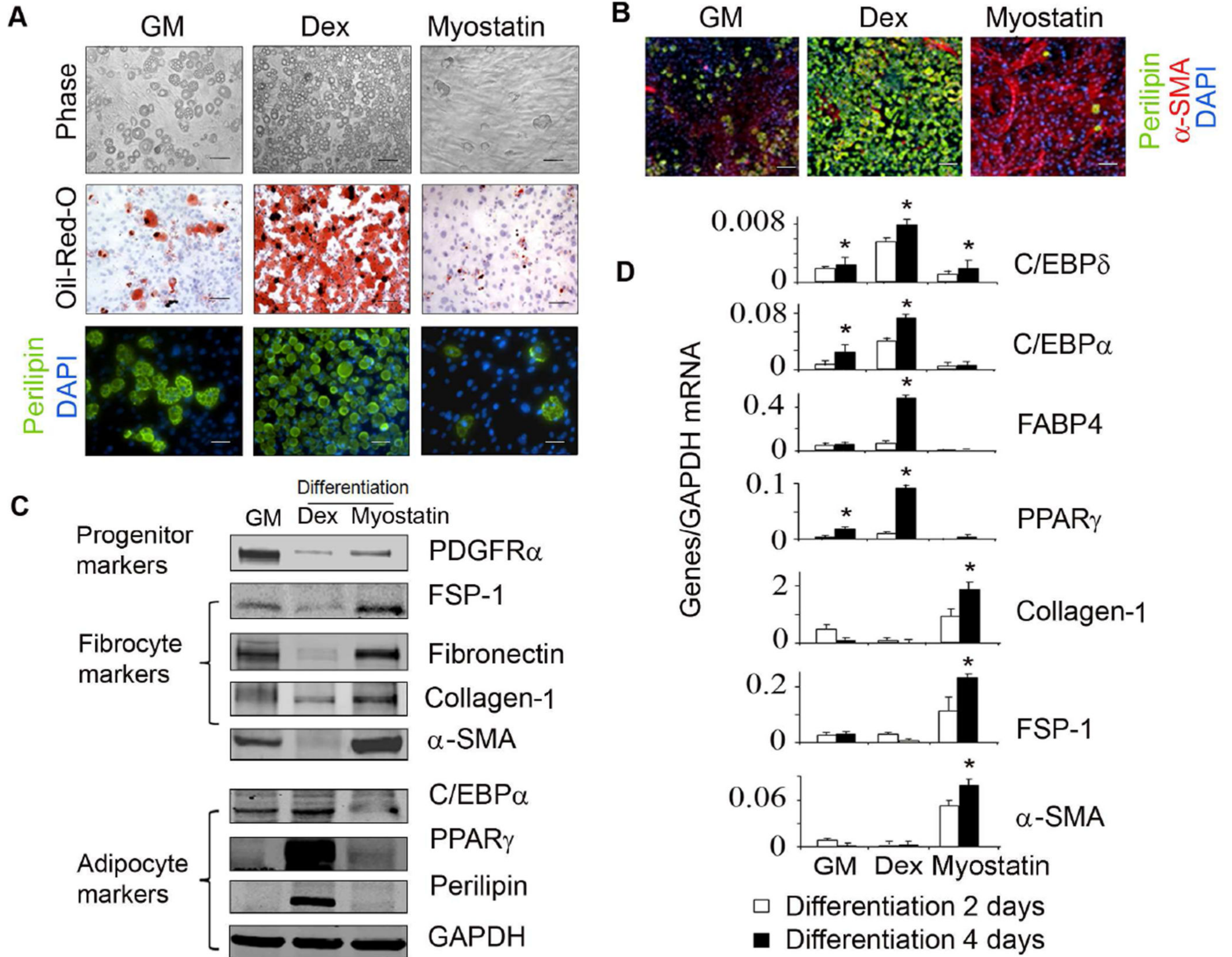

Figure 3. Myostatin induces fibrotic differentiation of FAPs

A. After 5 days of incubating FAPs in growth media, cells were switched to adipogenic differentiation media plus Dex or myostatin for 5 days (top panel), or 7 days stained with Oil-RedO (middle panel) or 14 days immunostained with anti-perilipin (bottom panel). B. After 4 days differentiation, FAPs were co-immunostained with anti-α-SMA (red) and anti-perilipin (green). C. FAPs cultured with adipogenic differentiation media plus Dex or myostatin for 4 days were lysed and subjected to western blotting. D. FAPs cultured in adipogenic differentiation media plus Dex or myostatin for 2 or 4 days, mRNAs were evaluated by RT-PCR. (n=3 repeats; *, $P<0.05$ vs. growth media). In FAPs, Dex increased adipogenic genes while myostatin increased fibrotic genes.

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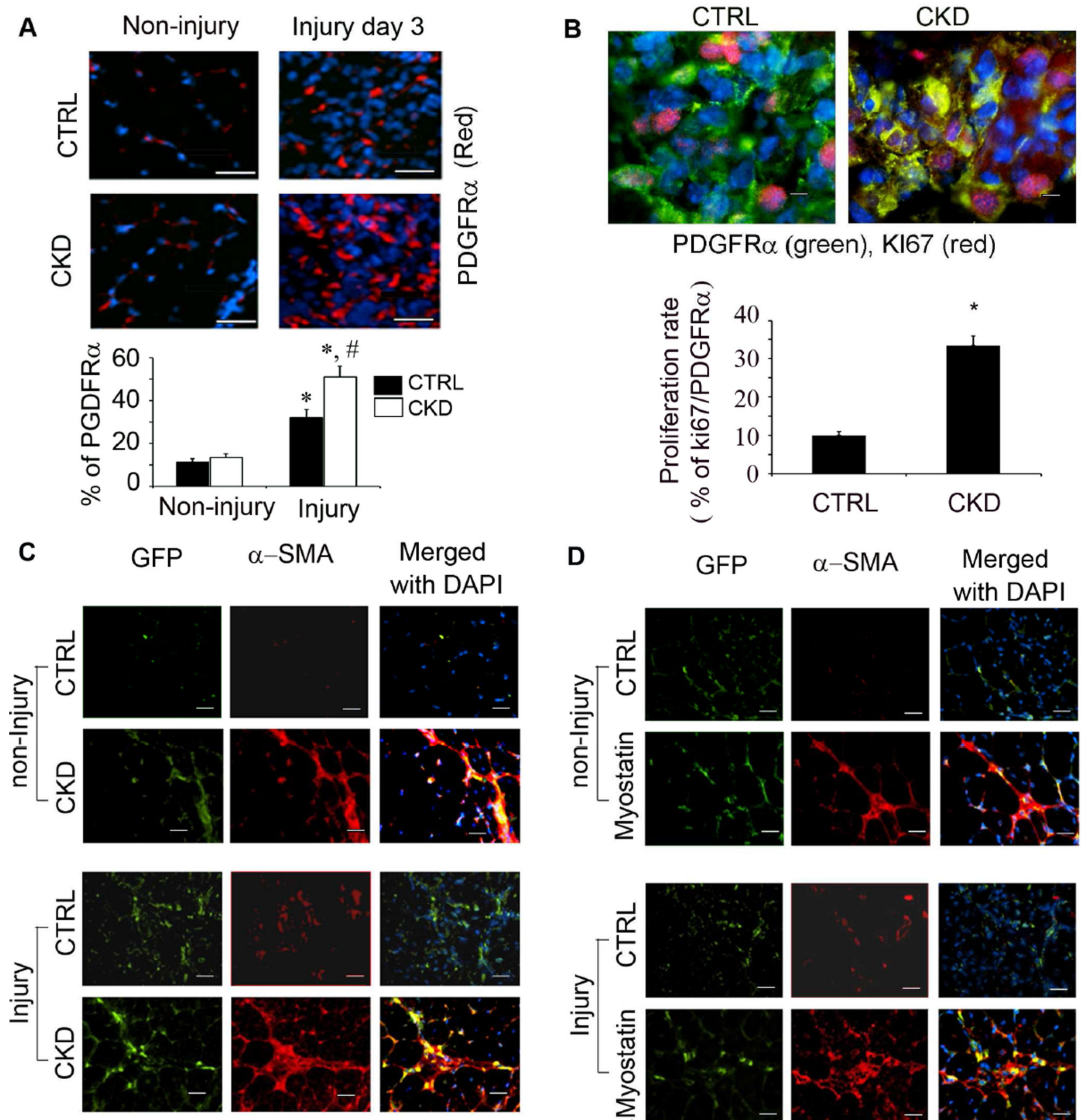

Figure 4. Fibrotic differentiation of FAPs in muscles of mice with CKD is mediated by myostatin

A. 3 days after muscle injury, sections were immunostained with anti-PDGFRα (red) and the percentage of PDGFRα$^+$ to total nuclear cells (DAPI) were counted (*, P<0.05 *vs.* control; #, P<0.05 *vs.* non-injury). CKD increased FAPs in muscle of CKD mice. B. Sections of injured muscles were co-immunostainned with anti- Ki67 and anti-PDGFRα. FAPs proliferation index is higher in injured muscles of CKD mice. C. FAPs-EGFP transplanted into TA muscles (with or without injury) of mice (with or without injury). After 7 days muscle injury and transplantation, sections of muscles were immunostained with anti-

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α-SMA. CKD or muscle injury increase muscle fibrosis. D. TA muscles were transplanted with FAPs-EGFP and simultaneously treated with myostatin. Sections immunostained with anti-α-SMA (Bar=50 μm) revealed that myostatin stimulated FAP conversion into fibrocytes.

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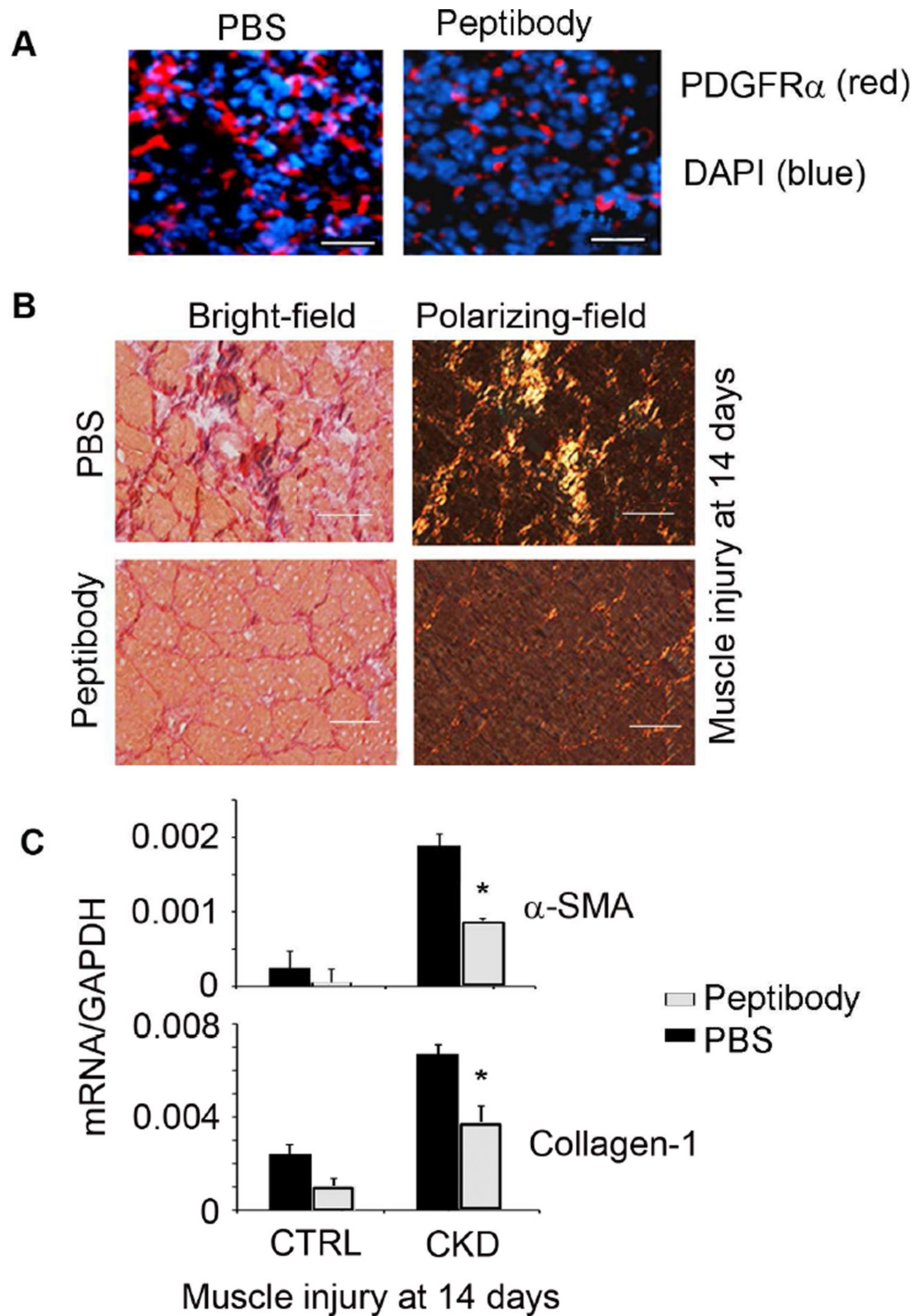

Figure 5. CKD induces fibrotic differentiation of FAPs via myostatin

A. Mice with CKD were treated with the anti-myostatin peptibody for 2 weeks. At 3 days after muscle injury, cryo-cross-sections of TA muscles were immunostained with anti-PDGFRα (Bar=50 μm). Myostatin inhibition decreased the number of PDGFRα$^+$ cells. B. At 14 days after muscle injury, peptibody treatment reduced muscle fibrosis. C. Peptibody treatment suppressed the mRNAs of fibrosis markers in muscles of CKD mice.

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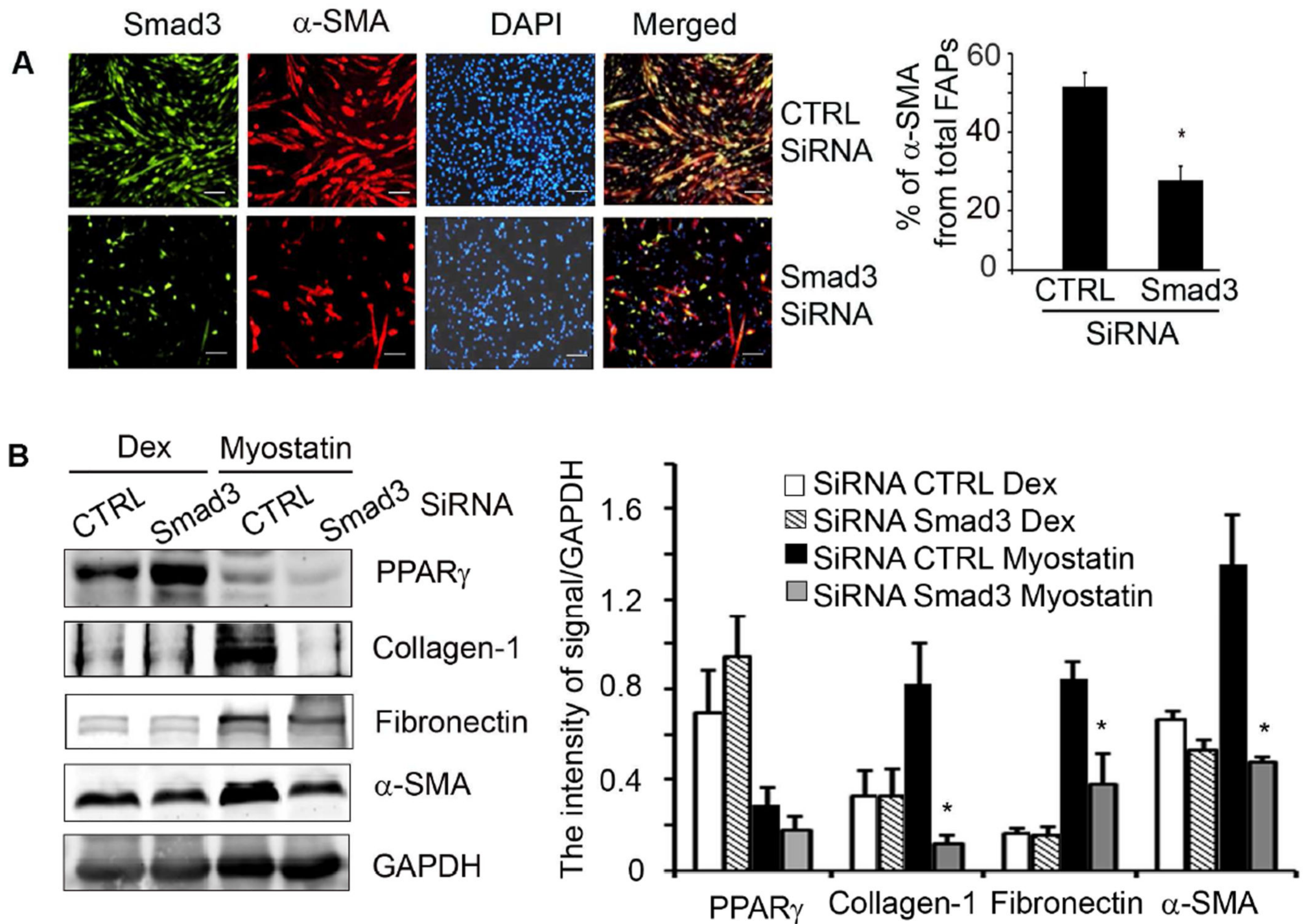

Figure 6. Myostatin induces fibrotic differentiation of FAPs via Smad3

A. Knockdown Smad3 in FAPs decreased myostatin-induced α-SMA cells (Bar=50 μm, *, P<0.05 vs SiRNA CTRL). B. Smad3- knocked down-FAPs cultured in media containing Dex or myostatin for 48h and reavealed that Smad3 knockdown in FAPs suppressed myostatin-induced fibrosis but not Dex-induced adipogenesis. (n=4 repeat; *, P<0.05 vs SiRNA of cells treated with myostatin).

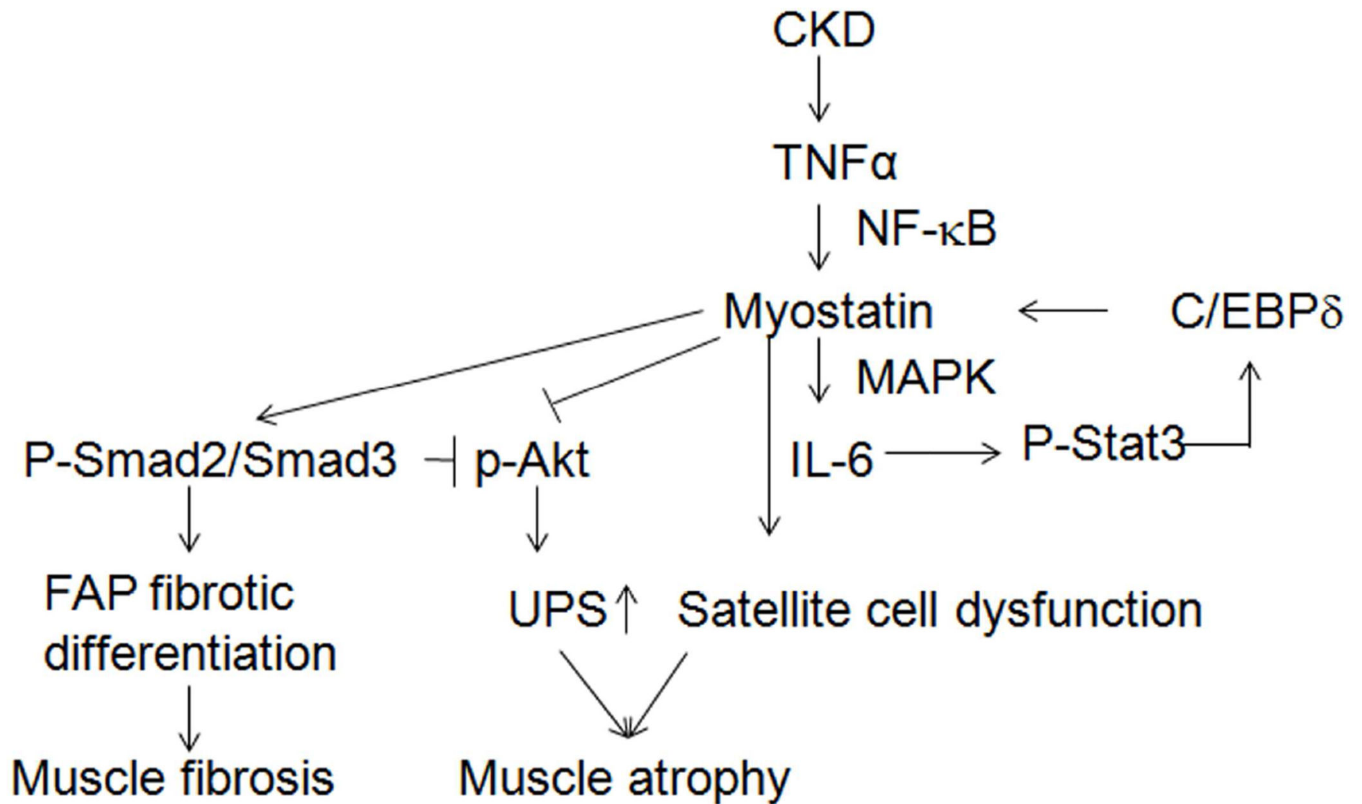

Figure 7. Mechanisms initiated by CKD cause atrophy and fibrosis in muscle

CKD-induced TNF-α stimulates myostatin production via NF-κB [22]. Myostatin production is augmented by Stat3 activation which stimulates the transcription factor, C/EBPδ [23]. The increase in myostatin impairs satellite cell function and contributes to the loss of muscle mass that is stimulated by CKD [1;22]. Myostatin raises Smad2/3 phosphorylation suppressing Akt phosphorylation resulting in activation of the ubiquitin-proteasome system (UPS) and muscle atrophy [22;34]. Finally, CKD causes muscle fibrosis because myostatin can convert FAPs into fibrocytes.