

ORIGINAL ARTICLE

Differentiation of skeletal muscle Mesenchymal progenitor cells to myofibroblasts is reversible

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Abstract

Accumulation of intramuscular adipose tissue (IMAT) and development of fibrous tissues due to accumulation of collagen both affect meat quality such as tenderness, texture, and flavor. Thus, it is important for the production of high-quality meat to regulate the amount of adipose and fibrous tissues in skeletal muscle. IMAT is comprised of adipocytes, while collagens included in fibrous tissues are mainly produced by activated fibroblasts. Both adipocytes and fibroblasts are differentiated from their common ancestors, called mesenchymal progenitor cells (MPC). We previously established rat MPC clone, 2G11 cells. As several reports implicated the plasticity of fibroblast differentiation, in the present study, using 2G11 cells, we asked whether myofibroblasts differentiated from MPC are capable of re-gaining adipogenic potential in vitro. By treating with bFGF, their α SMA expression was reduced and adipogenic potential was restored partially. Furthermore, by lowering cell density together with bFGF treatment, 2G11 cell-derived myofibroblasts lost α SMA expression and showed the highest adipogenic potential, and this was along with their morphological change from flattened- to spindle-like shape, which is typically observed with MPC. These results indicated that MPC-derived myofibroblasts could re-acquire adipogenic potential, possibly mediated through returning to an undifferentiated MPC-like state.

KEYWORDS

adipocyte, fibroblast, mesenchymal progenitor cell, skeletal muscle

1 | INTRODUCTION

The quality of meat is judged by some criteria, and the degree of intramuscular adipose tissue accumulation is one of the factors affecting meat quality such as tenderness, texture, and flavor (Wood et al., 1999). Besides, meat texture is also affected by the amount of fibrous tissues (Nishimura, 2010; Purslow, 2005). Type I collagen is one of the major collagen molecules consisting intramuscular

fibrous tissues, and the accumulation of excess amount of type I collagen decreases the texture and the value of meat (Nishimura, 2010; Purslow, 2005). Therefore, it is important for the production of high-quality meat to regulate the amount of adipose and fibrous tissues in skeletal muscle.

Several groups showed that platelet-derived growth factor receptor α (PDGFR α)-positive cells in interstitial space of mouse and human skeletal muscle differentiate into adipocytes and fibroblasts (Joe et al.,

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2010; Uezumi et al., 2011, 2014, 2010). These cells are called mesenchymal progenitor cells (MPC) (Uezumi et al., 2010) or fibrogenic/adipogenic progenitors (FAPs) (Joe et al., 2010). Fibrogenic/adipogenic differentiation of MPC is regulated by several growth factors. For example, induction of fibrogenic differentiation of MPC is induced by transforming growth factor (TGF)- β (Uezumi et al., 2011, 2014). We previously established a highly adipogenic cell clone, 2G11 cells, from rat skeletal muscle (Murakami et al., 2011), and showed that 2G11 cells also possess fibroblastic differentiation potential dependent on TGF- β (Takeuchi et al., 2016). Thus, 2G11 cells are considered to be a useful MPC clone to elucidate the regulatory mechanism involved in fibrogenic/adipogenic differentiation of MPC. By using 2G11 cells as a model of MPC, we also reported that basic fibroblast growth factor (bFGF) has a pro-adipogenic effect on MPC (Nakano et al., 2016). Therefore, it is possible that differentiation fate of MPC is regulated by the cross talk between TGF- β and FGF signals.

Activated fibroblasts (myofibroblasts) are main origin of extracellular matrix such as type I collagen (Takeuchi et al., 2016). Fibroblasts themselves express growth factors, such as TGF- β and connective tissue growth factor (CTGF), that are capable of inducing fibrogenic differentiation (Van Obberghen-Schilling, Roche, Flanders, Sporn, & Roberts, 1988; Takeuchi et al., 2016; Dorn et al., 2018), and these fibrogenic factors are shown to maintain their fibroblastic features (Dorn et al., 2018). Activated fibroblasts in several organs, such as lung and liver, strongly express α -smooth muscle actin (α -SMA) (Zhang, Gharaee-Kermani, Zhang, Karmioli, & Phan, 1996; Sun et al., 2016).

Several studies implicated the plasticity of fibroblast differentiation. For example, myofibroblasts had been shown to differentiate into adipocytes during wound healing of skin (Plikus et al., 2017). In heart, fibroblasts can transit to endothelial cells after acute ischemic cardiac injury (Ubil et al., 2014). From these studies, it is possible that myofibroblasts differentiated from MPC in skeletal muscle also shows plasticity, and re-differentiate to other cell types such as adipocytes.

In the present study, using 2G11 cells as a model of skeletal muscle MPC, we examined whether the myofibroblasts differentiated from MPC are capable of differentiating to adipocytes *in vitro*. Here we demonstrated that, although myofibroblasts derived from MPC do not show adipogenic potential, exposure to bFGF together with lowering the cell density restores their adipogenic potential.

2 | MATERIALS AND METHODS

2.1 | Cell culture and induction of fibroblastic and adipogenic differentiation

The rat MPC clone, 2G11 cells (Murakami et al., 2011), was usually maintained in Dulbecco's modified Eagle medium (Gibco, Life Technologies, Palo Alto, CA, USA) containing 10% fetal bovine serum, 100 U/mL penicillin, 100 μ g/mL streptomycin, 50 μ g/mL gentamicin (proliferating medium; PM) with bFGF (10 ng/ml) on poly-L-lysine and fibronectin-coated multi-well culture plates and culture dishes.

To induce fibroblastic differentiation, 2×10^4 cells/mL were cultured in PM containing 10 ng/ml TGF- β 1 (R&D Systems; cat. no. 240-B) for 3 days. To induce adipogenic differentiation, unless otherwise stated, 2×10^4 cells/mL cells were pre-treated with PM containing 10 ng/ml bFGF (cat. no. 233-FB; R&D Systems, Minneapolis, MN, USA) overnight, then cultured in adipogenic differentiation medium (PM containing insulin (1 μ g/mL), dexamethasone (0.1 μ g/mL), isobutylmethylxanthine (27.8 μ g/mL), and troglitazone (10 μ mol/L; kindly gifted by Daiichi-Sankyo Co. Ltd, Tokyo, Japan) for 2 days. The medium was then changed to PM containing insulin and troglitazone, and the cells were further cultured for another 2 days.

In the experiments where 2G11-derived myofibroblasts were used, they were trypsinized and re-plated at a density shown in the results. Typically, for cell culture, 24-well plate or 10 cm dish (Iwaki AGC Techno Glass Co., Ltd, Shizuoka, Japan) were used with 0.5 or 5 ml culture medium, respectively.

All of the culture experiments were performed in triplicates, and repeated for several times to ensure the reproducibility. Only representative data were shown.

2.2 | Immunocytochemistry

For immunocytochemistry using anti-proliferator-activated receptor gamma (PPAR γ), and anti-perilipin antibodies, cells were fixed in 4% paraformaldehyde (PFA)/ phosphate buffered-saline (PBS) for 15 min at room temperature. Then, they were washed three times with PBS, followed by blocking with 5% normal goat serum (NGS) containing 0.1% Triton X-100 (Sigma, St. Louis, MO, USA) in PBS for 20 min at room temperature. The cells were incubated overnight with primary antibodies at 4°C, and labeled with an Alexa Fluor-conjugated second antibody (1:500 dilution; Invitrogen (Thermo Fisher Scientific), Waltham, MA, USA). Nuclei were counterstained with Hoechst 33258. For the quantitative analysis of adipogenesis, five different fields were randomly chosen, and photographed with a digital camera (DP70; Olympus) using the 10 \times objective of a fluorescence microscope (BX50; Olympus, Tokyo, Japan). The number of PPAR γ -positive cells and the number of nuclei were counted and the proportion of PPAR γ -positive cells was calculated. The perilipin-positive area was quantified by calculating mean pixel measurements using ImageJ software (ver.1.47; National Institutes of Health, Bethesda, MD, USA). Primary antibodies and their species of origin were as follows: anti-PPAR γ (1:100; mouse, clone E-8, sc-7273; Santa Cruz Biotechnology, Dallas, TX, USA), and anti-perilipin (1:500; rabbit, clone D1D8, #9349; Cell Signaling Technology, Danvers, MA, USA). All antibodies were diluted with 5% NGS in PBS.

2.3 | Oil Red O staining

Cells were fixed in 4% PFA/PBS for 15 min at room temperature, and subsequently washed three times with PBS. Then, they were stained with Oil Red O (2:3 mixture of 0.5% w/v Oil Red O in 2-propanol and

distilled water) staining for 8 min, followed by washing three times with PBS. For the quantitative analysis of adipogenesis, five fields were randomly selected and observed using the 10× objective of a fluorescence microscope (BX50) and photographed with a digital camera (DP70). Mean pixel measurements were calculated using ImageJ.

2.4 | Immunoblotting

At the end of culture, cells were lysed directly in 1 × Laemmli buffer containing 2.5% β-mercapthoethanol, and incubated at 100°C for 3 min, then, centrifuged at 13,700g for 3 min. The supernatant was separated by electrophoresis using a 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel, followed by electroblotting onto polyvinylidene fluoride membranes. Non-specific binding was prevented by incubation with 5% skim milk/Tris-buffered saline with 0.1% Tween20. Anti-α-smooth muscle actin (α-SMA) antibody (1:4,000; mouse, clone 1A4, A2547; Sigma) was used to detect the target proteins, followed by incubation with a horseradish peroxidase-conjugated secondary antibody (1:50,000; goat, 115-035-003; Jackson ImmunoResearch Laboratory, West Grove, PA, USA). Stained bands were visualized using an ECL western blotting analysis system (GE Healthcare Life Sciences, Buckinghamshire, UK). Quantitative analysis was done with ChemiDoc XRS + with Image Lab Software (Bio-Rad, Hercules, CA, USA). The highest value of measurements was set at “1,” and relative intensities of other bands were calculated. Since the use of several loading controls such as β-actin, GAPDH, and vinculin was all unsuccessful to ensure the equal amount of protein loading due to the changes in their expression along with differentiation, the amount of protein loaded per lane was fixed at 5 μg based on the measurement by commercially available BCA assay kit (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan).

2.5 | Quantitative reverse transcription-mediated polymerase chain reaction (qRT-PCR)

Total RNA was extracted from 2G11 cells cultured with or without TGF-β for 3 days using TRIzol Reagent (Invitrogen), and cDNA was synthesized using Super Script II kit (Invitrogen). qPCR was performed on a Light Cycler 2.0 (Roche Diagnostics, Roche, Basel, Switzerland) with the Thunderbird SYBR qPCR Mix (TOYOBO, Osaka, Japan). For qPCR, the following primer sets were used: *TGF-β1*: forward, 5'- CCTGAAAGGGCTCAACAC-3'; reverse, 5'- CAGTTCTTCTCTGTGGAGCTGA-3'; *TGF-β2*: forward, 5'- AGTGGCAGCTTTTGCTC-3'; reverse, 5'- GTAGAAAGTGGCG GGATG-3'; *TGF-β3*: forward, 5'- GAAGAGGGCCCTGGACAC-3'; reverse, 5'- GCGCACACAGCAGTTCTC-3'; and hypoxanthine-guanine phosphoribosyltransferase (*Hprt*): forward, 5'-GACCGG TTCTGTCATGTCG-3'; reverse, 5'-ACCTGGTTCATCATCACTAAT CAC-3'. The expression of each gene was analyzed using the crossing-point method, and was normalized with that of *Hprt*.

2.6 | Statistical analysis

Data are expressed as means ± SE. Unpaired *t* tests and two-way analysis of variance followed by the Tukey-Kramer test were used to evaluate statistical differences between groups. *P* values less than 0.05 were considered statistically significant.

3 | RESULTS

3.1 | Myofibroblasts differentiated from 2G11 cells exhibit almost no adipogenic potential

2G11 cells are skeletal muscle MPC clone, and are capable of differentiating to adipocytes (Murakami et al., 2011). We previously reported that 2G11 cells differentiate to activated fibroblasts (myofibroblasts) by TGF-β treatment (Takeuchi et al., 2016). To investigate whether myofibroblasts differentiated from 2G11 cells are still capable of differentiating to adipocytes, 2G11 cells were treated with or without TGF-β (10 ng/ml) for 3 days to induce fibroblastic differentiation, then the cells were re-plated at density of 2 × 10⁴ cells/mL, and cultured in adipogenic differentiation medium. 2G11 cells without TGF-β treatment responded to adipogenic stimuli, resulting in the appearance of numerous numbers of Oil Red O- (Figure 1a,c) and PPARγ-positive cells (Figure 1a,d). On the other hand, the adipogenic potential was greatly diminished in myofibroblasts derived from TGF-β-treated 2G11 cells, as revealed by the significantly decreased number of Oil Red O- (*p* < .01; Figure 1a,c) and PPARγ-positive cells (*p* < .01; Figure 1a,d) compared to the cells without TGF-β treatment. The overall cell number did not differ regardless of TGF-β treatment (Figure 1b). These results indicate that myofibroblasts differentiated from 2G11 cells by TGF-β treatment exhibit almost no adipogenic potential.

3.2 | Myofibroblasts differentiated from 2G11 cells partially lose their fibroblastic feature and re-acquire adipogenic potential by bFGF treatment

Previous report demonstrated that bFGF treatment reverses the fibrogenic effect of TGF-β in adipose-derived mesenchymal stem cells (Desai, Hsia, & Schwarzbauer, 2014). First, we investigated whether fibroblastic feature of 2G11 cell-derived myofibroblasts is affected by exposure to bFGF. 2G11 cells were treated with TGF-β for 3 days to induce fibroblastic differentiation, then re-plated at density of 2 × 10⁴ cells/mL, and further cultured with or without bFGF (10 ng/ml) for 3 days. As previously reported (Takeuchi et al., 2016), 2G11 cells without any growth factor treatment expressed negligible amount of αSMA (Figure 2a,b). TGF-β-induced myofibroblasts cultured without bFGF showed upregulated αSMA expression, while it was reduced in the cells with bFGF treatment (Figure 2a,b). Then, we examined whether the downregulated αSMA expression in 2G11 cell-derived myofibroblasts coincides with re-acquirement of adipogenic potential. We compared

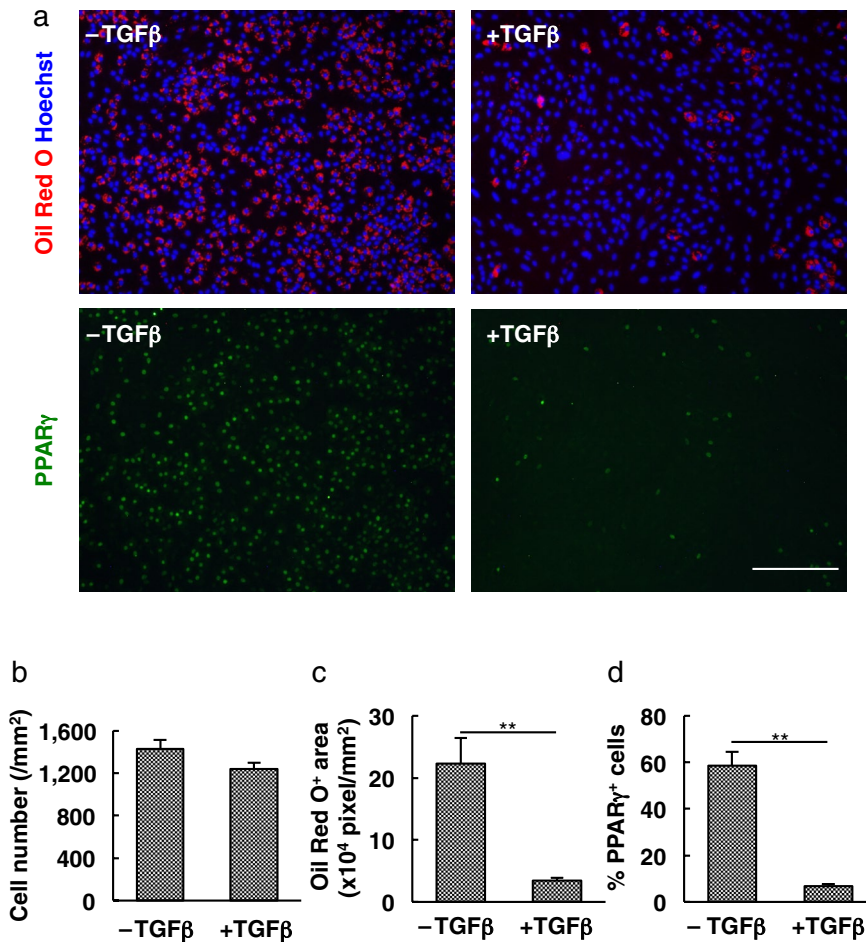


FIGURE 1 Adipogenic potential of 2G11 cell-derived myofibroblasts. (a) Oil Red-O staining (red) and immunocytochemistry of PPAR γ (green). Nuclei were stained with Hoechst 33258 (blue). Bar = 100 μ m. (b) Cell number at the end of culture in adipogenic differentiation medium. (c and d) Quantitative analyses of Oil Red O-positive area (c) and proportion of PPAR γ -positive cells (d) ($n = 3$). ** $p < .01$

the adipogenic potential between 2G11 cell-derived myofibroblasts treated with or without bFGF by means of immunocytochemistry of perilipin and PPAR γ . Typical staining pattern of perilipin and PPAR γ in 2G11 cell-derived adipocytes is shown in Figure S1. In agreement with the result shown in Figure 1, the adipogenic potential of 2G11 cells was strongly suppressed after TGF- β treatment as revealed by reduced staining of perilipin (Figure 2c,e) and PPAR γ (Figure 2c,f). On the other hand, the adipogenic potential of 2G11 cell-derived myofibroblasts was partially but significantly recovered by bFGF treatment as indicated by increased perilipin-positive area ($p < .01$; Figure 2c,e) and the proportion of PPAR γ -positive cells ($p < .01$; Figure 2c,f) compared to the cells without bFGF treatment. The cell number was not influenced regardless of the growth factor treatment (Figure 2d). These results indicate that 2G11 cell-derived myofibroblasts partially lose their fibroblastic feature, which is represented by α SMA expression, upon bFGF treatment, and re-acquire adipogenic potential.

3.3 | Lowering cell density enhances the re-acquirement of adipogenic potential in myofibroblasts differentiated from 2G11 cells by bFGF

Myofibroblasts are shown to express fibrogenic factors such as TGF- β , connective tissue growth factor (CTGF), and so on (Van

Obberghen-Schilling et al., 1988; Takeuchi et al., 2016; Dorn et al., 2018), and, by these factors, maintain their fibroblastic feature in an autocrine/paracrine manner (Dorn et al., 2018). We previously showed that TGF- β treatment induces CTGF expression in 2G11 cells (Takeuchi et al., 2016). Also, in the present study, 3 days TGF- β treatment on 2G11 cells significantly increased TGF- β 1, - β 2, and - β 3 expressions, respectively (Figure S2). Thus, it is possible that 2G11 cell-derived myofibroblasts also maintain their fibroblastic feature by these CTGF and TGF- β s in an autocrine/paracrine manner, and if so, reducing the cell density could lead to loss of their fibroblastic feature due to the lowered concentration of fibrogenic factors present in the culture medium. To test this possibility, 2G11 cell-derived myofibroblasts were re-plated at relatively high (2×10^5 cells/mL) and low (2×10^2 cells/mL) densities, then further cultured for 3 days. In addition, since 2G11 cell-derived myofibroblasts were shown to partially lose their fibroblastic feature upon bFGF treatment (Figures 1 and 2), we also examined whether the presence of bFGF (10 ng/ml) during the culture period after re-plating would have an additional effect on their fibroblastic feature. In the absence of bFGF treatment, the cell morphology of the cells cultured at high and low densities did not differ, and they both showed typical fibroblastic flattened shape (Figure 3a) although their α SMA expression was decreased in a cell density-dependent manner (Figure 3b). On the other hand, when the cells were cultured in the

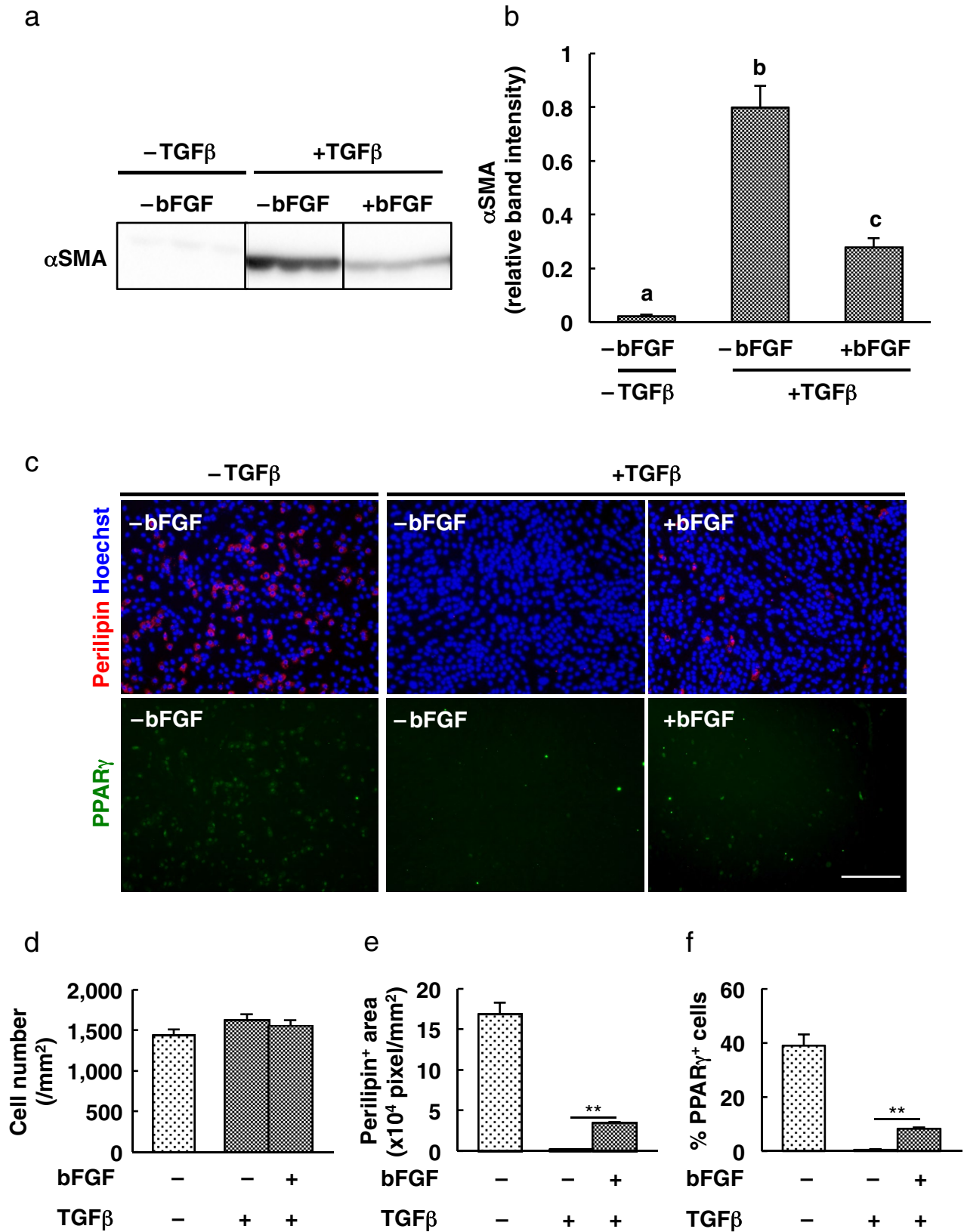


FIGURE 2 Effect of bFGF treatment on adipogenic potential of 2G11 cell-derived myofibroblasts. (a) Immunoblotting of α SMA. (b) Quantitative analysis of α SMA expression ($n = 3$). (c) Immunocytochemistry of perilipin (red) and PPAR γ (green). Nuclei were stained with Hoechst 33258 (blue). Bar = 100 μ m. (d) Cell number at the end of culture in adipogenic differentiation medium. (e and f) Quantitative analyses of proportion of perilipin-positive area (e) and PPAR γ -positive cells (f) ($n = 3$). ** $p < .01$

presence of bFGF at low density, almost all cells showed spindle-like shape (Figure 3a), which is typically seen in undifferentiated 2G11 cells. α SMA expression was decreased by the presence of bFGF, and at low density, it was almost undetectable (Figure 3b). The quantitative analysis between the cells cultured at high and low densities revealed that lowering cell density and bFGF-treatment have significantly additive effect in reducing α SMA expression ($p < .01$; Figure 3c).

The above results, that 2G11 cell-derived myofibroblasts cultured at low density in the presence of bFGF showed spindle-like shape and expressed negligible amount of α SMA, suggest that they returned to an undifferentiated state. Thus, we compared the adipogenic potential of 2G11 cell-derived myofibroblasts re-cultured at high and low

densities with or without bFGF. Even cultured at low density, 2G11 cell-derived myofibroblasts without bFGF treatment did not show adipogenic potential as revealed by staining of perilipin (Figure 4a,c) and PPAR γ (Figure 4a,d). On the other hand, the cells cultured at low density in the presence of bFGF exhibited the highest adipogenic potential ($p < .01$; Figure 4a,c,d). The overall cell number at the end of culture in adipogenic differentiation medium was slightly but significantly increased by culturing at low density (Figure 4). Taken together, these results suggest that 2G11 cell-derived myofibroblasts maintain their fibroblastic feature in an autocrine/paracrine manner, thus, they lose the feature by lowering cell density, and by bFGF treatment, they return to an undifferentiated state and re-acquire adipogenic potential.

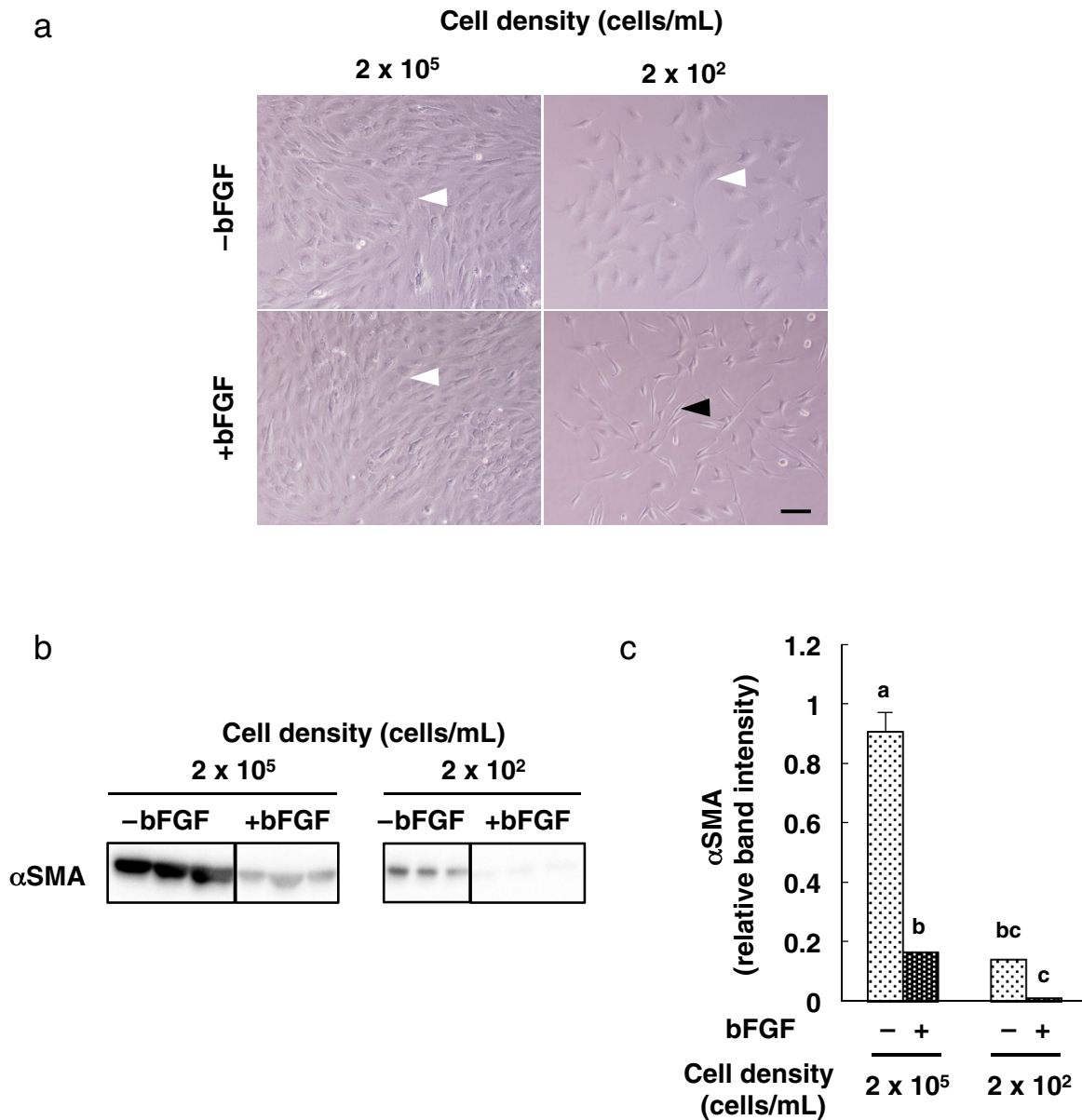


FIGURE 3 Effect of cell density together with bFGF treatment on cell shape and α SMA expression of 2G11 cell-derived myofibroblasts. (a) Phase contrast. White and black arrows indicate cells with flattened shape and spindle-like shape, respectively. (b) Immunoblotting of α SMA. (c) Quantitative analysis of α SMA expression ($n = 3$). Different letters indicate statistically significant difference ($p < .01$)

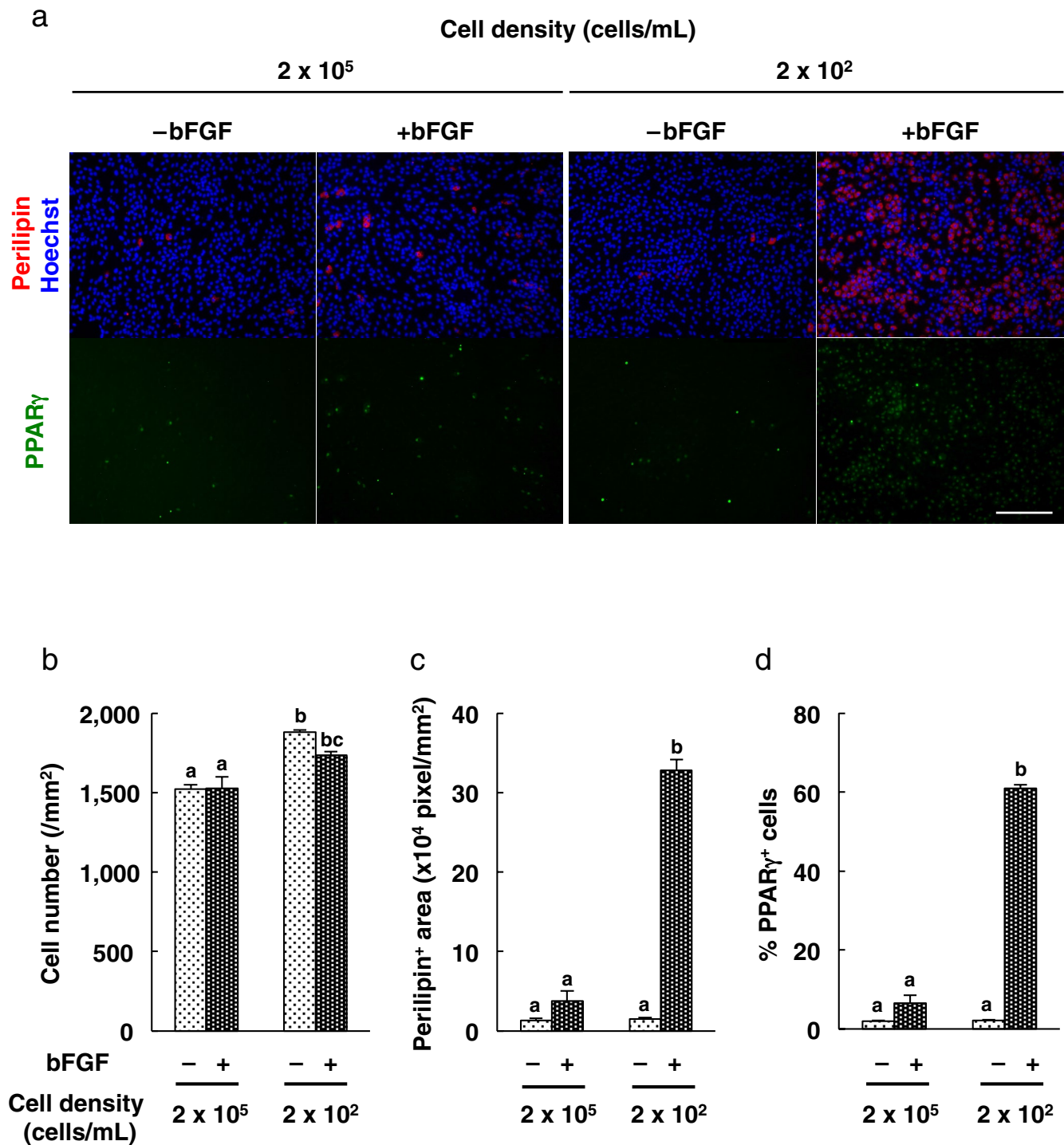


FIGURE 4 Effect of cell density together with bFGF treatment on adipogenic potential of 2G11 cell-derived myofibroblasts. (a) Immunocytochemistry of perilipin (red) and PPAR γ (green). Nuclei were stained with Hoechst 33258 (blue). Bar = 100 μ m. (b) Cell number at the end of culture in adipogenic differentiation medium. (c and d) Quantitative analyses of proportion of perilipin-positive area (c) and PPAR γ -positive cells (d) ($n = 3$). Different letters indicate statistically significant difference ($p < .01$)

4 | DISCUSSION

In the present study, we demonstrated that myofibroblasts differentiated from skeletal muscle MPC clone 2G11 cells are capable of returning to an undifferentiated MPC state, and re-acquiring adipogenic potential by lowering cell density in the presence of bFGF.

Several reports indicated the reversibility of activated fibroblasts (myofibroblasts) to their innate state (fibroblasts) depending on their extracellular environment. For example, in the fibroblasts from cornea, α SMA expression and the stress fiber formation, both are typical features of myofibroblasts, are changed depending on the cell density (Masur, Dewal, Dinh, Erenburg, & Petridou,

1996) and FGF signaling (Maltseva, Folger, Zekaria, Petridou, & Masur, 2001). Moreover, in lung fibroblasts, acidic FGF and prostaglandin E2 decreases the expression of α SMA (Ramos et al., 2006; Garrison et al., 2013). In the present study, by lowering the cell density, the α SMA expression in 2G11 cell-derived myofibroblasts was decreased, although the exact mechanism is unknown. Considering the expression of fibrogenic factors such as TGF- β s and CTGF by myofibroblasts themselves (Van Obberghen-Schilling et al., 1988; Takeuchi et al., 2016; Dorn et al., 2018), and the involvement of these factors to maintain their fibroblastic features in an autocrine/paracrine manner (Dorn et al., 2018), this might be due to the decreased amount of the fibrogenic factors present in culture. However, it should be noted that, despite the decreased α SMA expression, their typical fibroblastic flattened shape was maintained. In addition, by just lowering cell density, they did not re-gain adipogenic potential. This suggests that the fibrogenic factors produced from myofibroblasts are maintaining their fibroblastic feature, but its removal or decline would not lead them to further return to an undifferentiated MPC state.

It should be noted that, in the present study, regardless of the cell density, adipogenic potential was restored only when the myofibroblasts were treated with bFGF. Especially, the myofibroblasts cultured at low cell density with bFGF showed spindle-like shape, typically seen with undifferentiated MPC, and showed the highest adipogenic potential. CTGF expression is known to be induced by TGF- β signaling (Takeuchi et al., 2016), and bFGF has been shown to interfere SMAD pathway, which is located downstream of TGF- β signaling, in aortic valvular interstitial cells (Cushing et al., 2008), adipose-derived mesenchymal stem cells (Desai et al., 2014), and smooth muscle cells (Chen et al., 2016). Thus, one of the effects of bFGF on myofibroblasts is possibly counteracting the expression/action of fibrogenic factors such as TGF- β and CTGF. In addition to this bFGF action, we previously demonstrated that FGF signal is prerequisite for expressing adipogenic potential in 2G11 cells (Nakano et al., 2016). We routinely maintain 2G11 cells in culture in the presence of bFGF, and once bFGF is removed for several passages, they lose their adipogenic potential, but re-addition of bFGF for several days in culture restores the adipogenic potential (data not shown). Therefore, bFGF might also exert its effect not only in maintaining but also in restoring adipogenic potential in myofibroblasts. Furthermore, it was reported that the affinity between bFGF and FGF receptor is enhanced by lowering cell density in the primary corneal stromal fibroblasts (Richardson et al., 1999). Taken together, it is possible that, by culturing myofibroblasts at low density, bFGF action to restore the adipogenic potential is enhanced, leading to returning myofibroblasts to the undifferentiated state, harboring highly adipogenic potential.

In summary, we demonstrated for the first time that the activated fibroblasts (myofibroblasts) differentiated from skeletal muscle MPC could return to their undifferentiated state and re-acquire the adipogenic potential. Although it is currently unknown whether the similar situation does exist in vivo, the results of the present

study will be of value to establish the novel methodology to control meat quality in animal science field.

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REFERENCES

- Chen, P. Y., Qin, L., Li, G., Tellides, G., & Simons, M. (2016). Fibroblast growth factor (FGF) signaling regulates transforming growth factor beta (TGF β)-dependent smooth muscle 1cell phenotype modulation. *Scientific Reports*, 6, 1–11. <https://doi:10.1038/srep33407>
- Cushing, M. C., Mariner, P. D., Liao, J. T., Sims, E. A., & Anseth, K. S. (2008). Fibroblast growth factor represses Smad-mediated myofibroblast activation in aortic valvular interstitial cells. *The FASEB Journal*, 22, 1769–1777. <https://doi:10.1096/fj.07-087627>
- Desai, V. D., Hsia, H. C., & Schwarzbauer, J. E. (2014). Reversible modulation of myofibroblast differentiation in adipose-derived mesenchymal stem cells. *PLoS ONE*, 9, e86865. <https://doi.org/10.1371/journal.pone.0086865>
- Dorn, L. E., Petrosino, J. M., Wright, P., & Accornero, F. (2018). CTGF/CCN2 is an autocrine regulator of cardiac fibrosis. *Journal of Molecular and Cellular Cardiology*, 121, 205–211. <https://doi:10.1016/j.yjmcc.2018.07.130>
- Garrison, G., Huang, S. K., Okunishi, K., Scott, J. P., Penke, L. R. K., Scruggs, A. M., & Peters-Golden, M. (2013). Reversal of myofibroblast differentiation by prostaglandin E2. *American Journal of Respiratory Cell and Molecular Biology*, 48, 550–558. <https://doi:10.1165/rcmb.2012-0262OC>
- Joe, A. W. B., Yi, L., Natarajan, A., LeGrand, F., So, L., Wang, J., ... Rossi, F. M. V. (2010). Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. *Nature Cell Biology*, 12, 153–163. <https://doi:10.1038/ncb2015>
- Maltseva, O., Folger, P., Zekaria, D., Petridou, S., & Masur, S. K. (2001). Fibroblast growth factor reversal of the corneal myofibroblast phenotype. *Investigative Ophthalmology and Visual Science*, 42, 2490–2495.
- Masur, S. K., Dewal, H. S., Dinh, T. T., Erenburg, I., & Petridou, S. (1996). Myofibroblasts differentiate from fibroblasts when plated at low density. *Proceedings of the National Academy of Sciences of the United States of America*, 93, 4219–4223. <https://doi:10.1073/pnas.93.9.4219>
- Murakami, Y., Yada, E., Nakano, S.-i., Miyagoe-suzuki, Y., Hosoyama, T., Matsuwaki, T., Yamanouchi, K., & Nishihara, M. (2011). Establishment of bipotent progenitor cell clone from rat skeletal muscle. *Animal Science Journal*, 82(6), 764–772. <https://doi.org/10.1111/j.1740-0929.2011.00907.x>
- Nakano, S.-i., Nakamura, K., Teramoto, N., Yamanouchi, K., & Nishihara, M. (2016). Basic fibroblast growth factor is pro-adipogenic in rat skeletal muscle progenitor clone, 2G11 cells. *Animal Science Journal*, 87(1), 99–108. <https://doi.org/10.1111/asj.12397>
- Nishimura, T. (2010). The role of intramuscular connective tissue in meat texture. *Animal Science Journal*, 81(1), 21–27. <https://doi.org/10.1111/j.1740-0929.2009.00696.x>
- Plikus, M. V., Guerrero-Juarez, C. F., Ito, M., Li, Y. R., Dedhia, P. H., Zheng, Y., ... Cotsarelis, G. (2017). Regeneration of fat cells from

- myofibroblasts during wound healing. *Science*, 355(6326), 748–752. <https://doi.org/10.1126/science.aai8792>
- Purslow, P. P. (2005). Intramuscular connective tissue and its role in meat quality. *Meat Science*, 70(3), 435–447. <https://doi.org/10.1016/j.meatsci.2004.06.028>
- Ramos, C., Montañó, M., Becerril, C., Cisneros-Lira, J., Barrera, L., Lung Cellular and Molecular Physiology Lung Cellular and Molecular Physiology Ruíz, V., Pardo, A., & Selman, M. (2006). Acidic fibroblast growth factor decreases alpha-smooth muscle actin expression and induces apoptosis in human normal lung fibroblasts. *American Journal of Physiology Lung Cellular and Molecular Physiology*, 291, L871–879. <https://doi.org/10.1152/ajplung.00019.2006>
- Richardson, T. P., Trinkaus-Randall, V., & Nugent, M. A. (1999). Regulation of basic fibroblast growth factor binding and activity by cell density and heparan sulfate. *Journal of Biological Chemistry*, 274(19), 13534–13540. <https://doi.org/10.1074/jbc.274.19.13534>
- Sun, K. H., Chang, Y., Reed, N. I., & Sheppard, D. (2016). α -Smooth muscle actin is an inconsistent marker of fibroblasts responsible for force-dependent TGF β activation or collagen production across multiple models of organ fibrosis. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, 310(9), L824–L836. <https://doi.org/10.1152/ajplung.00350.2015>
- Takeuchi, S., Nakano, S.-I., Nakamura, K., Ozoe, A., Chien, P., Yoshihara, H., ... Nishihara, M. (2016). Roles of chondroitin sulfate proteoglycan 4 in fibrogenic/adipogenic differentiation in skeletal muscle tissues. *Experimental Cell Research*, 347(2), 367–377. <https://doi.org/10.1016/j.yexcr.2016.08.023>
- Ubil, E., Duan, J., Pillai, I. C. L., Rosa-Garrido, M., Wu, Y., Bargiacchi, F., ... Deb, A. (2014). Mesenchymal-endothelial transition contributes to cardiac neovascularization. *Nature*, 514(7524), 585–590. <https://doi.org/10.1038/nature13839>
- Uezumi, A., Ito, T., Morikawa, D., Shimizu, N., Yoneda, T., Segawa, M., ... Fukada, S. (2011). Fibrosis and adipogenesis originate from a common mesenchymal progenitor in skeletal muscle. *Journal of Cell Science*, 124(21), 3654–3664. <https://doi.org/10.1242/jcs.086629>
- Uezumi, A., Fukada, S., Yamamoto, N., ... Tsuchida, K. (2014). Identification and characterization of PDGFR α + mesenchymal progenitors in human skeletal muscle. *Cell Death & Disease*, 5, e1186. <https://doi.org/10.1038/cddis.2014.161>
- Uezumi, A., Fukada, S., Yamamoto, N., ... K. (2010). Mesenchymal progenitors distinct from satellite cells contribute to ectopic fat cell formation in skeletal muscle. *Nature Cell Biology*, 12(2), 143–152. <https://doi.org/10.1038/ncb2014>
- Van Obberghen-Schilling, E., Roche, N. S., Flanders, K. C., Sporn, M. B., & Roberts, A. B. (1988). Transforming growth factor beta 1 positively regulates its own expression in normal and transformed cells. *The Journal of Biological Chemistry*, 263, 7741–7746.
- Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Richardson, R. I., & Sheard, P. R. (1999). Manipulating meat quality and composition. *Proceedings of the Nutrition Society*, 58, 363–370.
- Zhang, H. Y., Gharaee-Kermani, M., Zhang, K., Karmioli, S., & Phan, S. H. (1996). Lung fibroblast alpha-smooth muscle actin expression and contractile phenotype in bleomycin-induced pulmonary fibrosis. *The American Journal of Pathology*, 148(2), 527–537.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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