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A Dissertation
Submitted to the Department of Surgery and the
Graduate School of Yonsei University
in partial fulfillment of the requirements for
the degree of
Doctor of Medicine in Surgery

Seongyup Kim

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Contents

Abstract

Skeletal muscle satellite cells (SkMSCs) play crucial roles in muscle fiber maintenance,repair, and remodeling; however, it remains unknown if these properties are preserved in cultured SkMSCs. In this study, we investigated the characteristics of cultured SkMSCs and their ability to regulate the activity of M1 macrophages. SkMSCs grew well with an average population doubling time of 26.26 ± 6.85 h during 10 passages (P). At P5, Pax7, MyoD, cluster of differentiation (CD)34, and CD56 were not expressed in SkMSCs, but the MSC markers CD73, CD105, and CD90 were expressed and the cells were differentiated into adipocytes and osteoblasts. When SkMSCs were cocultured with macrophages, interleukin (IL)-1β secretion was decreased, prostaglandin (PG)E2 was produced in coculture, and cyclooxygenase-2 protein was induced in an SkMSC-dependent manner. Hepatocyte growth factor (HGF) was highly secreted by monocultured SkMSCs; interferon-γ and lipopolysaccharide reduced its expression level. However, HGF expression recovered when SkMSCs and macrophages were cocultured. Although exogenous PGE2 upregulated macrophage pro-IL-1β expression, it suppressed the secretion of cleaved IL-1β. In contrast, HGF decreased active IL-1β secretion without affecting pro-IL-1β expression. Co-treatment of macrophages with HGF and PGE2 reduced pro-IL-1β expression level and active IL-1β secretion. Our results suggest that

SkMSCs lose their satellite cell properties during serial passaging but acquire mesenchymal stem cell properties including the ability to exert an anti-inflammatory response for macrophages through PGE2 and HGF.

INTRODUCTION

Although skeletal muscle satellite cells (SkMSCs) play an effector role during the regeneration of damaged muscle, other resident cell types, including motor neurons, endothelial cells, immune cells, and fibroadipogenic progenitors (FAPs, play important roles in muscle repair and homeostasis (Heredia et al., 2013; Joe et al., 2010; Lukjanenko et al., 2019; Tatsumi et al., 2009). In response to muscle injury, the acute inflammatory response is initiated by neutrophil infiltration followed by infiltration of M1 macrophages (Otis et al., 2014; Schneider & Tiidus, 2007), which leads to SkMSC activation (Tidball, 2017) and extracellular matrix production by FAPs (Lemos et al., 2015). M1 macrophages infiltrating injury sites produce inflammatory cytokines, including tumor necrosis factor-α, interleukin (IL)-1β, and interferon (IFN)-ɣ (Mosser & Edwards, 2008). These cytokines increase the proliferation of primary myoblasts, suggesting that M1 macrophages participate in SkMSC activation (Otis et al., 2014; Palacios et al., 2010). In addition to their myogenic potential, SkMSCs exhibit mesenchymal plasticity (Seale et al., 2008;

Shefer et al., 2004). Cells expressing the myogenic regulatory factor Myf5 specifically gave rise to skeletal myoblasts and brown fat cells through the transcriptional regulator PRD1-BF1-RIZ1 homologous domain containing 16 (Seale et al., 2008). In addition, clonal analyses of SkMSCs showed that Pax7-expressing progenitors can commit to mutually exclusive myogenic or mesenchymal alternative differentiation pathways (Shefer et al., 2004).

MATERIALS AND METHODS

Cell culture

This study was approved by the Institutional Review Board of Yonsei University Wonju College of Medicine (CR320308). SkMSCs at passage (P) 1 were purchased from ScienCell (Cat no. 3510) and subcultured in a poly-L-lysine (ScienCell)-coated culture vessel and skeletal muscle cell medium (ScienCell) supplemented with 2% fetal bovine serum (FBS), antibiotics, and skeletal muscle cell growth supplement (ScienCell). Cryopreserved primary cells were seeded in 100-mm dishes, and the medium was replaced the next day to remove any residual dimethyl sulfoxide and unattached cells. Once the cells reached 90% confluency, they were continuously subcultured until P10 to examine their proliferation potential. The population doubling time was determined by dividing the total number of hours of culture by the number of doublings. P5

cells were used for this experiment.

THP-1 cells (Cat no. 40202; Korean Cell Line Bank) were maintained in Roswell Park Memorial Institute-1640 medium (Gibco BRL) supplemented with 10% FBS, antibiotics, and 2mM L-glutamine. Macrophage differentiation of THP-1 cells was induced by adding 100 nM of phorbol ester 12-O-tetradecanoylphorbol-13-acetate (Sigma-Aldrich) and culturing the cells for 2 days; the cells were either monocultured or cocultured with SkMSCs in Transwell plates (Corning, Inc.) and treated with 20 ng/ml IFN-γ (R&D Systems) and 10 pg/ml lipopolysaccharide (LPS; Sigma-Aldrich). Conditioned medium was collected, filtered (0.45 μm), and stored at −80° C until analysis of active IL-1β, HGF, and PGE2.

Next-generation sequencing (NGS)

Total RNA was extracted from 1 × 105 cells using TRIzol Reagent (Gibco BRL) according to the manufacturer's instructions. Libraries were prepared for 150-bp paired-end sequencing using a TruSeq stranded mRNA Sample Prep Kit (Illumina). The messenger RNA (mRNA) samples were purified and fragmented from 1 μg of total RNA using oligo(dT) magnetic beads. These fragmented mRNAs were synthesized as single-stranded complementary DNAs (cDNAs) using random hexamer primers, and then used as templates for second strand synthesis and to prepare double-stranded cDNA. After sequential end repair, A-tailing, and adapter ligation, the cDNA libraries were amplified by polymerase chain reaction. The

quality of these cDNA libraries was evaluated using an Agilent 2100 BioAnalyzer (Agilent Technologies), and the cDNA libraries were quantified using a KAPA Library Quantification Kit (Kapa Biosystems) according to the manufacturer's instructions. Following cluster amplification of denatured templates, the paired-end reads (2 × 150 bp) were sequenced using an Illumina NovaSeq. 6000 (Illumina). Low-quality reads were filtered according to the following criteria: reads with more than 10% skipped bases, reads with more than 40% of bases with quality scores<20, and average quality scores <20 for each read. The filtering process was performed using in-house scripts.

Adipogenic or osteogenic differentiation

For adipogenic differentiation, SkMSCs (2 × 104 cells/cm2) were added to six-well plates and cultured for 1 week. The medium was then changed to adipogenic medium (Dulbecco's Modified Eagle Medium supplemented with 10% FBS, 1 μM dexamethasone, 0.5mM 3-isobutyl-1-methylxanthine, 10 μg/ml insulin, and 100 μM indomethacin) for an additional week. The cells were then fixed in 4% paraformaldehyde for 10 min. To evaluate adipogenic differentiation, fresh Oil Red O solution was used to stain the lipid droplets in the cells, which were then photographed. Oil Red O was then eluted with isopropanol, and the extracted Oil Red O was quantified by measuring the absorbance at 540 nm.

For osteogenic differentiation, the cells (2 × 104 cells/cm2) were seeded into six-well plates and cultured in osteogenic medium (Dulbecco's Modified Eagle Medium supplemented with 10% FBS, 10mM β-glycerophosphate, 10-7M dexamethasone, and 0.2mM ascorbic acid) for 2 weeks. To determine the osteogenic differentiation potential, calcium deposits, which are indicators of mature osteocytes, were stained with 2% Alizarin Red S (ScienCell); the stain was then extracted with 10% (v/v) acetic acid according to the manufacturer's instructions. Differentiation was quantified by measuring the absorbance at 405 nm.

Surface antigen expression

A total of 5 × 105 SkMSCs at P5 was stained with phycoerythrin-conjugated antibodies against CD34, CD73, CD90, CD105, human leukocyte antigen-DR (BD Biosciences), and CD140α (Invitrogen), fluorescein-conjugated antibody against CD15 (BD Biosciences), and allophycocyanin-conjugated antibody against CD56 (BD Biosciences) at room temperature for 20 min. Mouse IgGs were used as the isotype control. The fluorescence intensity of the cells was evaluated using flow cytometry (FACS Aria III; BD Biosciences).

Immunoblotting

Proteins were separately prepared from cells and conditioned medium. The cells were lysed in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (62.5mM Tris-HCl, pH 6.8; 1% SDS; 10% glycerol; and 5% β-mercaptoethanol), and the conditioned medium was mixed (1:1) with 2× sample buffer. Protein samples were boiled for 5 min, subjected to SDS-PAGE, and transferred to an Immobilon polyvinylidene difluoride membrane (Millipore). The membrane was blocked with 5% skim milk in Tris-HCl buffered saline containing 0.05% Tween-20 and then incubated with primary antibodies against IL-1β, cleaved IL-1β (1:2000; Cell Signaling Technology), COX-2, and GAPDH (1:1000; Santa Cruz Biotechnology), followed by peroxidase-conjugated secondary antibodies (1:2000; Santa Cruz Biotechnology). The membrane was treated with EZ-Western Lumi Pico or Femto (DOGEN) and visualized using a ChemiDoc XRS + system (Bio-Rad).

Enzyme-linked immunosorbent assay (ELISA)

The conditioned medium was used to detect the secretion of active IL-1β, PGE2, and HGF. The concentrations of active IL-1β, PGE2, and HGF were measured using a human IL-1β ELISA Kit II (R&D Systems), PGE2 Parameter Assay Kit (R&D Systems), and human HGF Quantikine ELISA Kit (R&D Systems), respectively, according to the manufacturer's instructions.

Statistical analysis

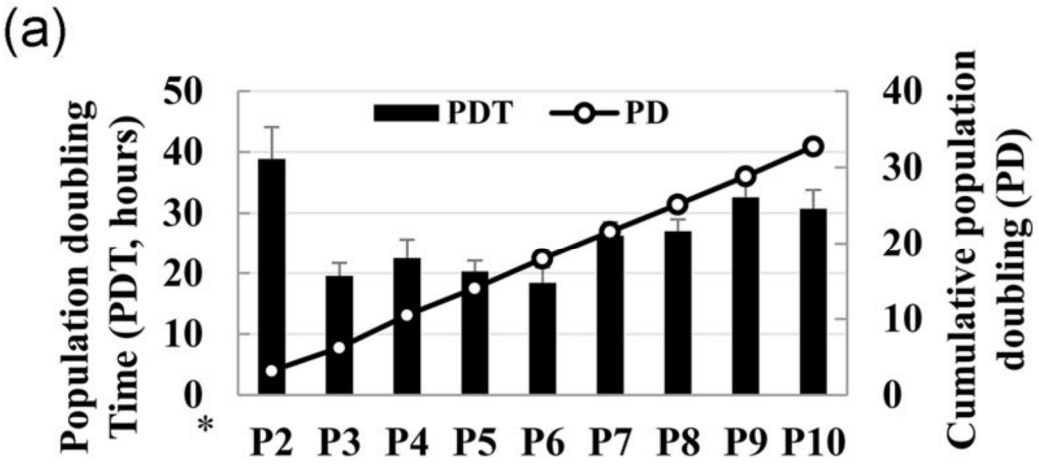
Data are presented as the mean ± SD of the mean. To compare the group means, Student's t test and one-way analysis of variance were performed, followed by Scheffe's test. $p < .05$ was considered significant.

RESULTS

Characteristics of cultured SkMSCs at P5

To confirm whether the cultured SkMSCs maintained the characteristics of satellite cells, the expression of the myogenic cell markers CD56, Pax7, and MyoD and proliferation potential was examined. In serial subculture up to P10, except for P2, the population doubling time was maintained at around 20-30 h, and the total number of population doublings was 32.7 (Figure 1a). The transcriptional factors Pax7 and MyoD showed low expression in SkMSCs at P5 (Figure 1b). Additionally, SkMSCs at P5 did not express CD56, a cell surface antigen of satellite cells, and expression of CD15 and CD140, which are markers of FAPs, was observed in 9.5 ± 0.8% and 42.8 ± 11.5% of cells, respectively (Figure 1c). These results suggest that cultured SkMSCs lose the characteristics of satellite cells but are converted into fibroblastlike cells

that differ from FAPs. Next, the ability of cultured SkMSCs and MSCs to differentiate into adipocytes and osteoblasts as well as expression of cell surface antigens were compared. Cultured SkMSCs differentiated into adipocytes and osteoblasts, showing a higher differentiation efficiency than MSCs (Figure 1d). The cell surface markers expressed by SkMSCs at P5 were CD73 (97.1 ± 4.5%), CD90 (38.1 ± 18.4%), and CD105 (99.0 ± 0.2%), but CD34 and human leukocyte antigen-DR (HLA-DR) were not expressed (Figure 1e). These results suggest that cultured SkMSCs have very similar differentiation capacities and surface marker expression as MSCs.



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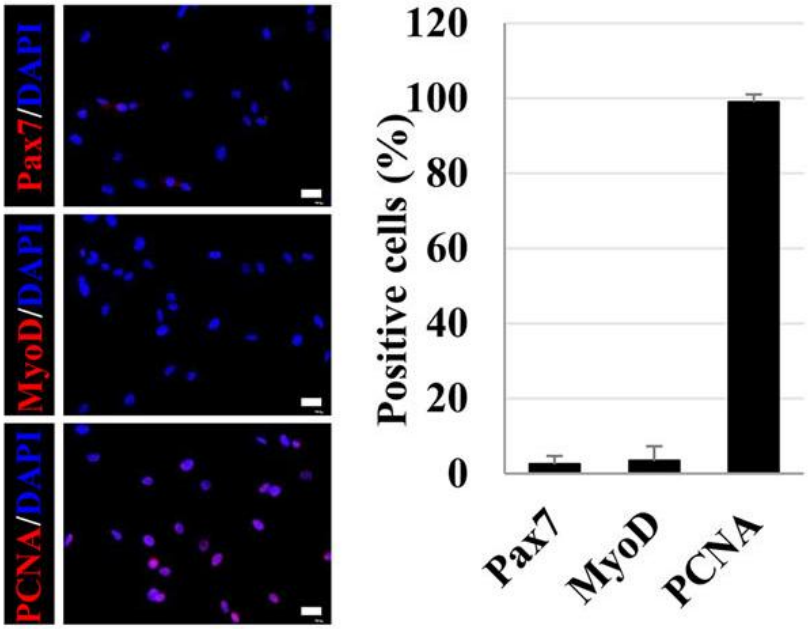

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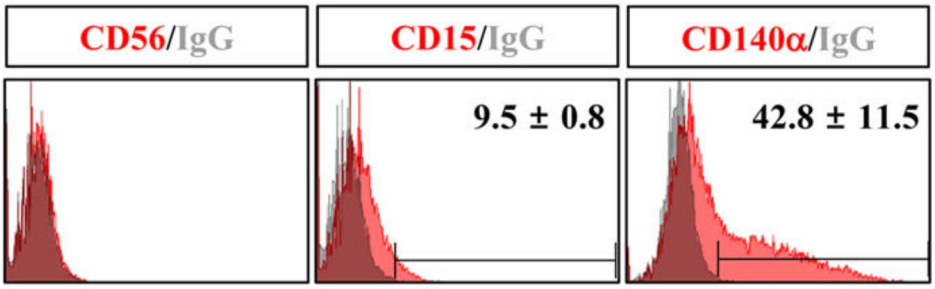


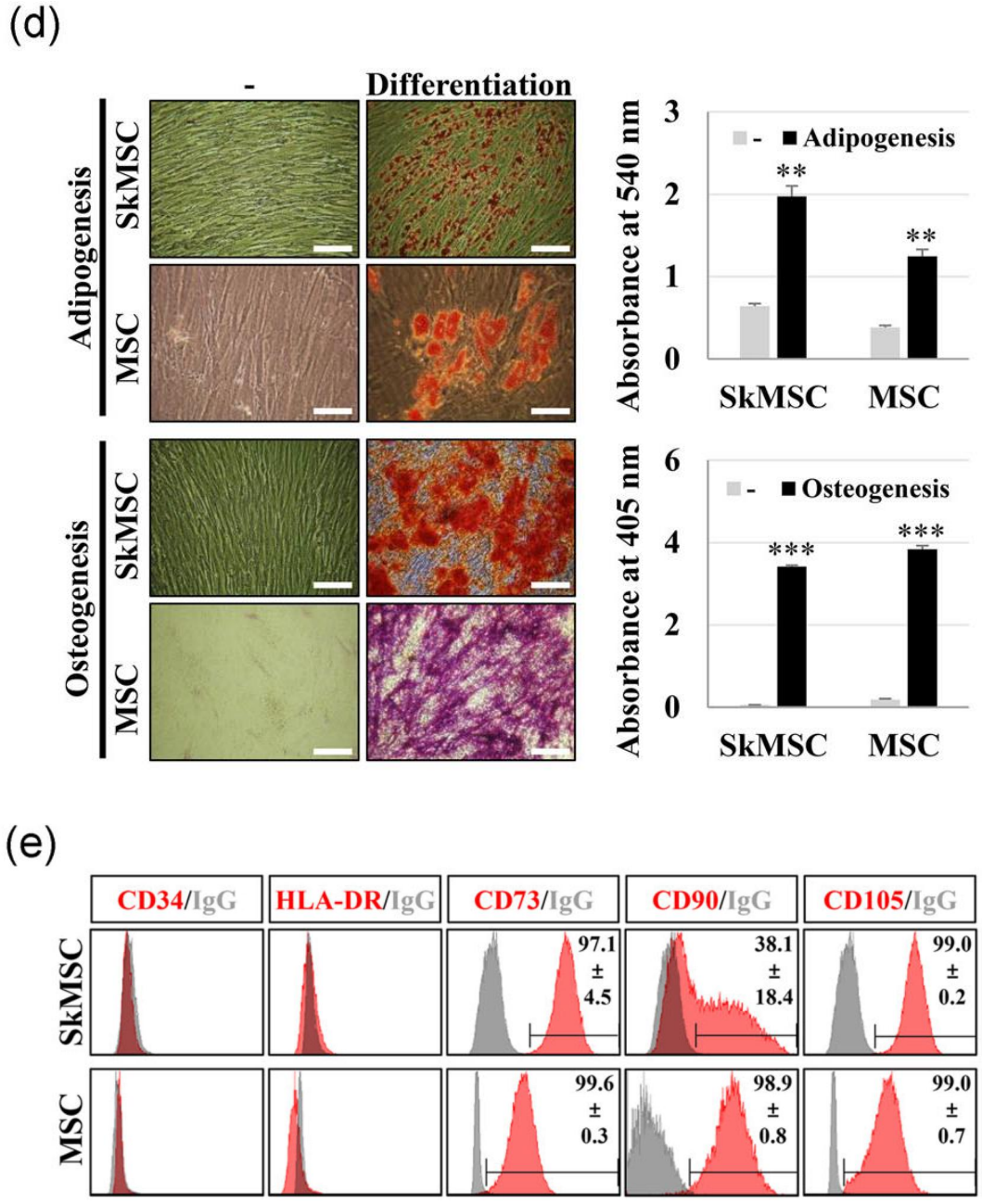


FIGURE 1 Characteristics of cultured skeletal muscle satellite cells (SkMSCs) at passage (P)5. SkMSCs were serially cultured until P10, after which transcriptional markers, cell surface markers, and differentiation potential

were evaluated. (a) Proliferation potential of SkMSCs. SkMSCs were serially passaged until P10, and the population doubling time and number of population doublings were calculated to define the proliferation potential. (b) Expression of Pax7 and MyoD in SkMSCs. SkMSCs at P5 was immunocytochemically stained with Pax7 and MyoD antibodies. The SkMSCs did not express Pax7 or MyoD, but almost all cells were proliferating cell nuclear antigen (PCNA)-positive. Scale bar = 20 μm. (c) Expression of cell surface antigens against SkMSCs (i.e., CD56) or fibro-adipogenic progenitors (FAPs) (i.e., CD15 and CD140α). Data were obtained from one of three independent experiments. Positive cell populations are shown as the mean ± SD from three independent experiments. (d) Adipogenic and osteogenic differentiation potential of SkMSCs and mesenchymal stem cell (MSCs). Data were obtained from one of three independent experiments. **p ≤ .01; ***p < .001 (n = 3). Scale bar = 100 μm. (e) MSC-cell surface antigen expression of SkMSCs and MSCs. Data were obtained from one of three independent experiments. Positive cell populations are shown as the mean ± SD of three independent experiments

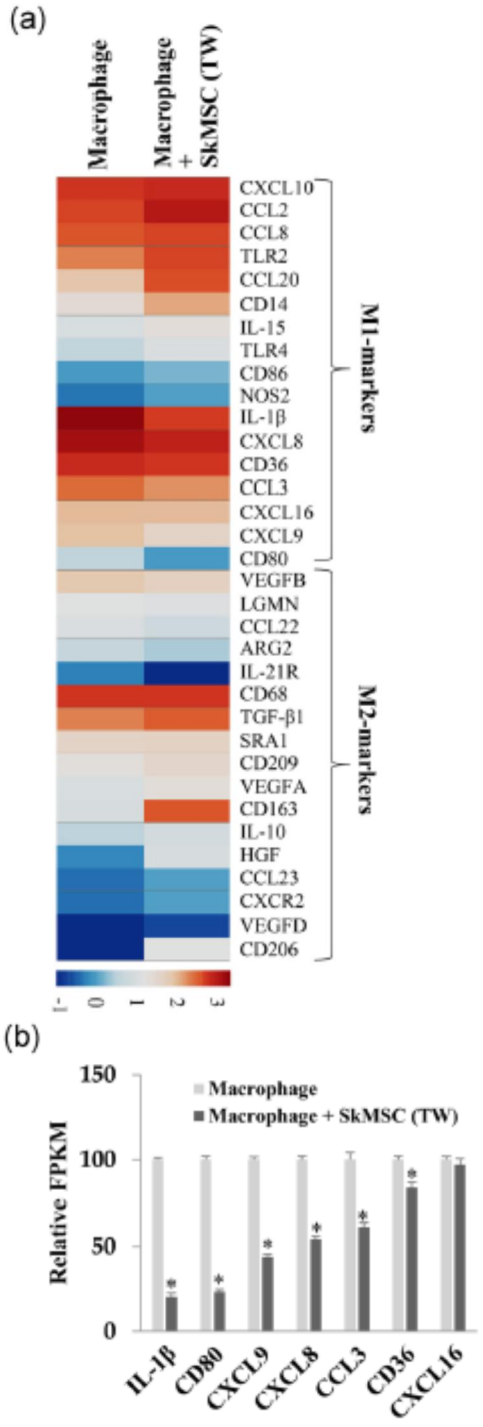
Phenotypic changes of macrophages by SkMSCs

To understand the crosstalk between cultured SkMSCs and macrophages, we investigated whether SkMSCs could modulate macrophage phenotypes. Phorbol ester 12-O-tetradecanoylphorbol-13-acetateinduced THP-1 cells (macrophages) were treated with IFN-γ and LPS in an indirect coculture system with or without SkMSCs for 2 days. Next, the levels of macrophage markers were evaluated by NGS. After indirect coculture of macrophages and SkMSCs, the expression levels of M1 macrophage markers, including IL-1β, CD80, chemokine (C-X-C motif) ligand (CXCL)9, CXCL8, chemokine (C-C motif) ligand (CCL)3, CD36, and CXCL16, were decreased (Figure 2a,b). Conversely, among the M2 markers, the expression of CD163, HGF, CXCR2, vascular endothelial growth factor (VEGF)A, IL-10, CCL23,

VEGFD, CD209, transforming growth factor (TGF)-β1, and steroid receptor RNA activator 1 (SRA1) was increased (Figure 2a,c). In addition, the expression of CD206, a surface marker of M2 macrophages, was increased in macrophages cocultured with SkMSCs (Figure 2d). These results suggest that SkMSCs can induce a shift in macrophage polarization from M1 to M2 phenotype, similar to MSCs (Park et al., 2018).

Suppressive effect of SkMSCs on expression and secretion of IL-1β

Previously, we reported that MSCs suppressed inflammatory cytokine IL-1β secretion by M1 macrophages via PGE2 (Park et al., 2018). To determine whether cultured SkMSCs also inhibit the secretion of IL-1β from macrophages, SkMSCs at P5 were indirectly cocultured with IFN-γ- and LPS-treated macrophages in a transwell system for 2 days, and the expression level of pro-IL-1β in the cell lysate and active IL-1β in the culture supernatants was examined. IFN-γ and LPS induced pro-IL-1β expression in macrophages, but this expression was reduced upon coculture with SkMSCs (Figure 3a). In addition, the secretion of cleaved IL-1β in macrophages was reduced by SkMSCs (Figure 3b). These results suggest that cultured SkMSCs can suppress the activities of IL-1β by downregulating pro-IL-1β expression and inhibiting active IL-1β secretion.



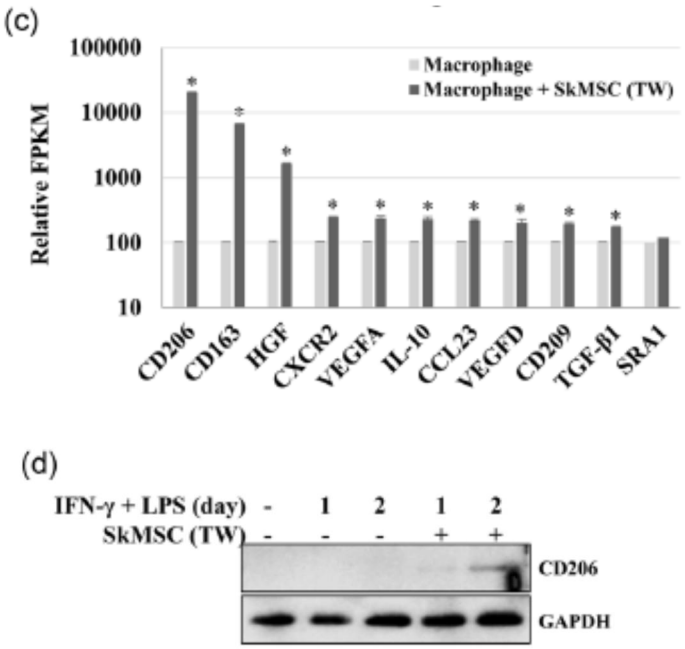


FIGURE 2 Phenotypic changes in macrophages induced by cultured skeletal muscle satellite cells (SkMSCs). SkMSCs were cocultured with macrophages for 2 days; M1 and M2 macrophage markers were then analyzed by next-generation sequencing (NGS). (a) Heatmap representation of the relative M1 and M2 marker expression levels. Among these, M1 markers whose expression was decreased by SkMSCs and M2 markers whose expression was increased by SkMSCs were selected; their relative expression was then compared in monocultured macrophages and macrophages cocultured with SkMSCs. (b) Decreased expression of M1 markers upon coculture with SkMSCs. (c) Increased expression of M2 markers upon coculture with SkMSCs. The relative fragments per kilobase million (FPKM) values were normalized against GAPDH. Error bars represent the mean ± SD from triplicate analysis. *$p \leq .05$ ($n = 3$). LPS, lipopolysaccharide; TW, transwell

Inhibitory effects of PGE2 and HGF on IL-1β expression and secretion in macrophages

In a previous study, MSCs were found to enhance the expression of cellular pro-IL-1β in macrophages but inhibit active IL-1β secretion (Park et al.,

2018); in contrast, SkMSCs inhibited both cellular pro-IL-1β expression and active IL-1β secretion in macrophages (Figure 3). Therefore, we considered that, in addition to PGE2, other factors may be involved in regulating IL-1β activity in macrophages. Particularly, we focused on HGF, which showed significantly increased expression in macrophages during their coculture with SkMSCs (Figure 2). In addition, HGF is known to be involved in skeletal muscle regeneration through macrophage transition to the M2 phenotype and in decreasing IL-1β expression in rat macrophages (Choi et al., 2019). First, during macrophage mono-culture and their coculture with SkMSCs, we analyzed the expression levels of HGF and cyclooxygenase-2 (COX-2) through NGS. COX-2 is themain enzyme that produces PGs (e.g., PGD2, PGE2, PGI2, PGF2α, thromboxane A2) in each cell type, and PGE2 is the main product of COX-2. The fragments per kilobase million values of COX-2 and HGF in macrophages were 0.84 ± 0.24 and 0.13 ± 0.08, respectively; however, after IFN-γ and LPS treatment for 2 days, these values were increased to 2.59 ± 0.85 and 0.47 ± 0.47, respectively. Moreover, macrophages cocultured with SkMSCs showed a significant increase in COX-2 and HGF levels, which were 10.85 ± 1.32 and 7.67 ± 1.15, respectively (Figure 4a). However, COX-2 protein levels in the macrophages were not increased, even under SkMSC coculture conditions (Figure 4c). Notably, monocultured SkMSCs did not express COX-2 but expressed a high level of HGF mRNA (Figure 4b). In SkMSCs exposed to IFN-γ and LPS, COX-2 mRNA increased,

whereas HGF mRNA decreased (Figure 4b). In addition, in SkMSCs cocultured with macrophages, both the mRNA expression and protein levels of COX-2 were increased (Figure 4b,d). PGE2 production was detected only in cocultured SkMSCs and macrophages, and its concentration was approximately 2.6 ng/ml (Figure 4e). HGFs were detected in the culture supernatant obtained from monocultured SkMSCs, with or without IFN-γ and LPS treatment, as well as in the coculture of SkMSCs and macrophages. Approximately 3.4 ng/ml of HGF was detected in the conditioned medium obtained from SkMSCs, but its level was decreased to approximately 1.1 ng/ml by IFN-γ and LPS treatment. However, the HGF level was recovered to 2.2 ng/ml by coculture of SkMSCs and macrophages (Figure 4e). Taken together, SkMSCs expressed HGF at a high level (3.4 ng/ml), and both PGE2 and HGF were produced in coculture of SkMSCs and macrophages.

Next, we investigated whether both PGE2 and HGF regulated IL-1β expression and secretion in macrophages. IFN-γ and LPS increased the expression of pro-IL-1β and secretion of active IL-1β in macrophages. PGE2 elevated the expression of pro-IL-1β but inhibited the secretion of active IL-1β. HGF decreased the active IL-1β secretion by more than PGE2 without affecting the expression of pro-IL-1β. Co-treatment with PGE2 and HGF significantly reduced both pro-IL-1β expression and active IL-1β secretion (Figure 5). Thus, PGE2 and HGF can regulate IL-1β activity by downregulating pro-IL-1β expression and active IL-1β secretion.

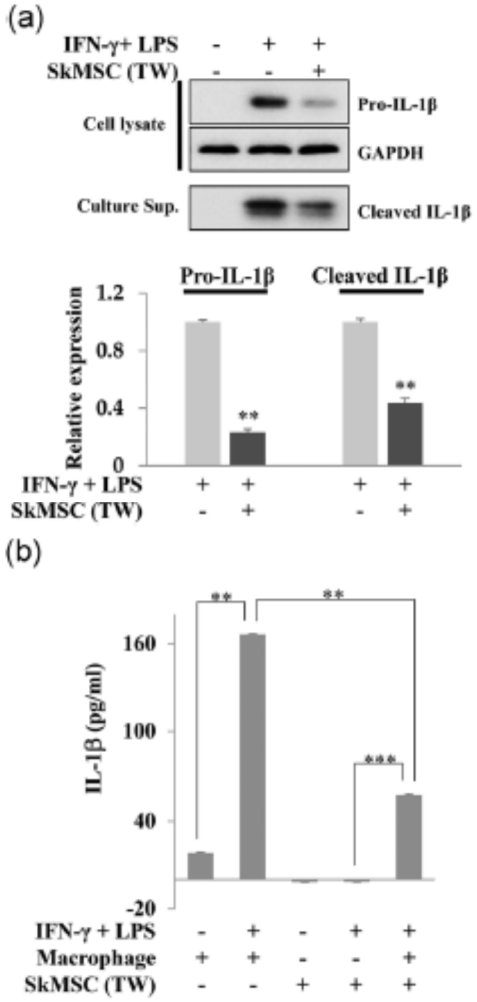


FIGURE 3 Regulation of IL-1β expression and secretion by skeletal muscle satellite cells (SkMSCs) in macrophages. To analyze the effect of SkMSCs on IL-1β regulation, cellular pro-IL-1β expression and active IL-1β secretion was investigated in monocultured macrophages and macrophages cocultured with SkMSCs. (a) Expression of pro-IL-1β and cleaved IL-1β in macrophages. Error bars represent the mean ± SD from triplicate analysis. Data were obtained from one of three independent experiments. **p ≤ .01 (n = 3). (b) Active IL-1β secretion levels. Under each culture condition, active IL-1β was detected in the culture supernatant by enzyme-linked immunosorbent assay (ELISA). Data represent the mean ± SD from three independent experiments. **p ≤ .01 (n = 3). LPS, lipopolysaccharide; TW, transwell

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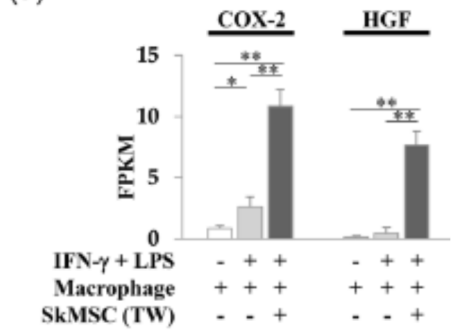

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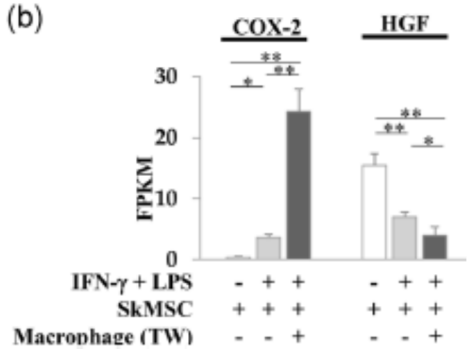

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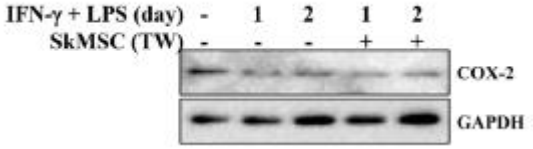

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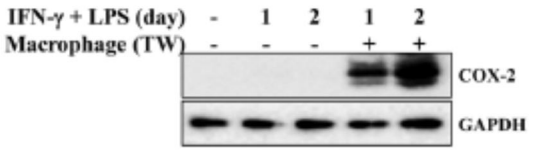


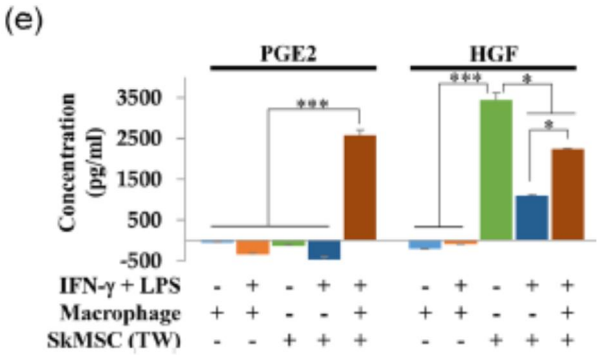
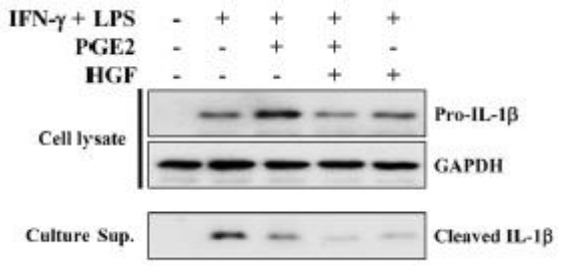


FIGURE 4 Expression of COX-2 and hepatocyte growth factor (HGF) in skeletal muscle satellite cells (SkMSCs) and macrophages. Monocultured SkMSCs and macrophages were treated with IFN-γ and LPS, or SkMSCs and macrophages were indirectly cocultured under IFN-γ and LPS treatment for 2 days. The messenger RNA (mRNA) levels of COX-2 and HGF were analyzed by next-generation sequencing, and their protein levels were detected by immunoblotting or nzyme-linked immunosorbent assay (ELISA), respectively. (a,b) COX-2 and HGF mRNA expression in macrophages (a) and SkMSCs (b). Expression levels are represented as fragments per kilobase million values obtained by next-generation sequencing analysis. Error bars represent the mean ± SD of triplicate analysis. *p ≤ .05; **p ≤ .01 (n = 3). (c,d) COX-2 protein expression in macrophages (c) and SkMSCs (d). Data were obtained from one of three independent experiments. (e) Production of PGE2 and HGF. Data represent the mean ± SD of three independent experiments. *p ≤ .05; ***p < .001 (n = 3)



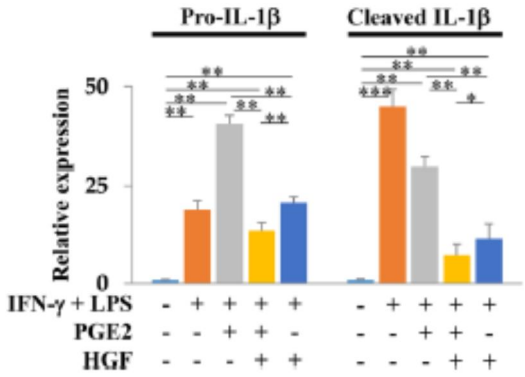


FIGURE 5 Inhibitory effects of PGE2 and hepatocyte growth factor (HGF) on IL-1β expression and secretion. Macrophages were treated exogenously with PGE2 (2.5 μM) and HGF (1 ng/ml), and then pro-IL-1β expression was detected in the cell lysates and active IL-1β secretion was determined in the culture supernatant by immunoblotting. Data were obtained from one of three independent experiments. Error bars represent the mean ± SD of triplicate analysis. *p ≤ .05; **p ≤ .01; ***p < .001 (n = 3). LPS, lipopolysaccharide

DISCUSSION

We observed that cultured SkMSCs lost their satellite cell properties but acquired MSC properties, such as adipogenic and osteogenic differentiation capabilities, cell surface marker expression (i.e., CD73, CD90, and CD105), and an anti-inflammatory response for macrophages via PGE2 and HGF. We also observed that SkMSCs decreased the expression level of M1 markers and increased that of M2 markers in macrophages, thereby leading to M2 transition of macrophages.

The differentiation period of SkMSCs into osteoblasts was 2 weeks, similar to the 2-3 weeks of MSCs but the differentiation period of SkMSCs into adipocytes

was only 1 week, much shorter than that of MSCs (Eom et al., 2014; Pittenger et al., 1999). Previously, we reported that HGF is important in maintaining the adipogenic differentiation of MSCs (Eom et al., 2014) and observed that high levels of HGF were expressed by the SkMSCs (Figure 4). Therefore, the adipogenic differentiation period of SkMSCs expressing HGF is expected to be shorter than that of MSCs.

PGE2 is a key molecule modulating inflammation and was increased in the coculture of macrophages and adipose tissue-derived MSCs (ASCs), thus increasing M2 transition and the suppression of macrophage-dependent inflammation (Park et al., 2018). ASCs increased pro-IL-1β levels but suppressed the secretion of active IL-1β in macrophages through PGE2 (Park et al., 2018). However, although the same macrophages were used, the increase in PGE2 after coculture of SkMSCs and macrophages did not increase pro-IL-1β expression to more than that in macrophages cocultured with ASCs (Park et al., 2018) or in cells treated with exogenous PGE2 (Figure 5). These results suggest that both ASCs and SkMSCs inhibit the activity of M1 macrophages, but their inhibitory mechanisms may differ.

Further, Choi et al. (2019) reported that HGF regulated the macrophage transition to the M2 phenotype by activating AMPK signaling and decreasing LPS-induced IL-1β, iNOS, and tumor necrosis factor-α levels in Raw 264.7 cells. They showed that inhibition of c-Met signaling delayed muscle regeneration,

whereas exogenous delivery of HGF facilitated muscle repair in a cardiotoxin-induced muscle injury model. In our experiment, exogenous HGF also reduced IL-1β secretion in human macrophages. HGF interacts with its cellular receptor, c-met, and plays a multifunctional role in the regeneration of various tissues, including the skeletal muscle, through mitogenic, morphogenic, motogenic, and angiogenic activities (Huh et al., 2004; Nakamura & Mizuno, 2010). In addition, HGF was reported to promote the activation of quiescent satellite cells in injured muscles and in in vitro culture to enter the cell cycle (Tatsumi et al., 1998), as well as to stimulate the migration of myoblasts in vitro (Fibbi et al., 2002). After muscle injury, the levels of HGF mRNA and protein were increased (Jennische et al., 1993); its expression level in macrophages was increased sevenfold compared with that in non-macrophage cells (Sisson et al., 2009). Similarly, the expression of HGF mRNA in macrophages cocultured with SkMSCs was increased 60-fold compared with that in monocultured macrophages. Notably, normal SkMSCs expressed high levels of HGF; however, HGF expression was significantly decreased by IFN-γ and LPS and/or macrophages. Further, HGF mRNA expression in macrophages cocultured with SkMSCs increased by approximately 60-fold compared with that in monocultured macrophages, whereas HGF expression in the cocultured SkMSCs decreased by fourfold compared with that in monocultured SkMSCs. Thus, although macrophages were found to

FIGURE 5 Inhibitory effects of PGE2 and hepatocyte growth factor (HGF) on IL-

1β expression and secretion. Macrophages were treated exogenously with PGE2 (2.5 μM) and HGF (1 ng/ml), and then pro-IL-1β expression was detected in the cell lysates and active IL-1β secretion was determined in the culture supernatant by immunoblotting. Data were obtained from one of three independent experiments. Error bars represent the mean ± SD of triplicate analysis. *p ≤ .05; **p ≤ .01; ***p < .001 (n = 3). LPS, lipopolysaccharide be mainly responsible for the expression of HGF protein, SkMSCs were also considered as an important cell source expressing HGF.

In summary, cultured SkMSCs showed MSC-like phenotypes including anti-inflammatory effects on macrophages through PGE2 and HGF, leading to inhibition of IL-1β secretion. PGE2 and HGF were detected in the coculture of SkMSCs and macrophages, and exogenous treatment with PGE2 and HGF suppressed IL-1β activities by downregulating pro-IL-1β expression and active IL-1β secretion. In addition to macrophages, SkMSCswere found to be an important cell source of HGF. Taken together, the cultured SkMSCs were HGF-expressing cells and mitigated the inflammatory response of macrophages, similar to MSCs.
Our findings suggest that SkMSCs can function as immunomodulators to ameliorate muscle damage at muscle injury sites, as observed for MSCs.

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