Effect of Porcine Skeletal Muscle Satellite Cells on Lipid Deposition in Intramuscular Preadipocytes in a Co-culture System: Postprint

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Abstract

This study aimed to investigate the effects of porcine skeletal muscle satellite cells on lipid deposition in intramuscular preadipocytes within a co-culture system. Porcine skeletal muscle satellite cells and intramuscular preadipocytes were isolated and cultured. Following isolation, culture, and identification of both cell types, they were seeded into Transwell insert co-culture plates for co-cultivation. Upon reaching a cell density of over 90%, they were induced to differentiate separately. After 8 days of induced differentiation of skeletal muscle satellite cells, the differentiation level of intramuscular preadipocytes, the expression of differentiation marker genes, and the expression of key enzymes in lipid metabolism were examined. The results demonstrated that, compared with intramuscular preadipocytes cultured alone, the number and area of lipid droplets in intramuscular preadipocytes within the co-culture system were extremely significantly reduced ($P<0.01$). Additionally, the gene and protein expression levels of the proliferation and differentiation transcription factors peroxisome proliferator-activated receptor and CCAAT/enhancer-binding protein were extremely significantly decreased ($P<0.01$), as were the gene and protein expression levels of acetyl-CoA carboxylase and fatty acid synthase in intramuscular preadipocytes ($P<0.01$).

Full Text

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Abstract

This study aimed to investigate the effects of porcine skeletal muscle satellite cells on lipid deposition in intramuscular preadipocytes within a co-culture system. Porcine skeletal muscle satellite cells and intramuscular preadipocytes were isolated and cultured separately, then seeded into Transwell co-culture plates after identification. Once cell density exceeded 90%, differentiation was induced in both cell types. After 8 days of skeletal muscle satellite cell differentiation, the differentiation level of intramuscular preadipocytes, expression of differentiation marker genes, and key enzymes in lipid metabolism were assessed. The results demonstrated that compared with intramuscular preadipocytes cultured alone, the co-cultured intramuscular preadipocytes exhibited significantly reduced lipid droplet number and area ($P<0.01$). Additionally, the gene and protein expression levels of peroxisome proliferator-activated receptor (PPAR) and CCAAT enhancer binding protein (C/EBP), key transcription factors for preadipocyte proliferation and differentiation, were markedly decreased ($P<0.01$). Similarly, the gene and protein expression levels of acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) were significantly downregulated ($P<0.01$). These findings indicate that porcine skeletal muscle satellite cells exert an inhibitory effect on lipid deposition in intramuscular preadipocytes within the co-culture system.

Keywords: porcine skeletal muscle satellite cells; intramuscular preadipocytes; co-culture; lipid deposition

Introduction

Vertebrate muscle and adipocytes originate from a common mesodermal lineage and maintain intimate regulatory connections through autocrine and paracrine signaling during animal growth and development [1-2]. Investigating the interactions between adipose and muscle tissues is crucial for elucidating the mechanisms by which cytokines, nutritional factors, and hormones regulate fat deposition and muscle development. Furthermore, understanding these interactions has broad applications in human metabolic disease research and in improving meat quality traits in livestock.

To explore the mutual influences between adipose and muscle tissues during organismal development, researchers have established various cell culture systems.

Dodson et al. [3] first developed an in vitro co-culture system combining 3T3-L1 preadipocytes with ovine muscle cells at different differentiation stages. Because co-culture systems better simulate the physiological environment in vivo compared with monoculture, they have gained widespread attention among scholars. In recent years, investigators have established adipose-muscle cell co-culture systems in sheep, humans, mice, quail, and cattle to examine tissue interactions during development [4-8]. This study isolated and cultured porcine skeletal muscle satellite cells and intramuscular preadipocytes, established an indirect co-culture system, and investigated the proliferation and differentiation characteristics of both cell types. Specifically, we examined how porcine muscle cell differentiation regulates adipocyte differentiation and intracellular lipid deposition in the indirect co-culture system, providing a theoretical foundation for further revealing the interactions between adipose and muscle tissues.

Materials and Methods

1.1 Experimental Materials Reagents included 0.25% trypsin, 0.02% ethylenediaminetetraacetic acid (EDTA) (used at 1:1 ratio, Sigma, USA), trypan blue, 0.2% collagenase, cell cryopreservation medium, DMEM/F12 medium (Gibco, USA), fetal bovine serum (Gibco, USA), trypsin, phosphate-buffered saline (PBS) (powder, Beijing Solarbio Science & Technology), insulin, dexamethasone, premium horse serum (Gibco, USA), penicillin-streptomycin solution (100×, Beijing Solarbio), 3-isobutyl-1-methylxanthine (IBMX) (Sigma, USA), Oil Red O, Taco™ DNA/RNA extraction kit (Ruiji Marine Biotechnology), PrimeScript™ II 1st Strand cDNA Synthesis Kit (TaKaRa, Japan), All-in-One™ qPCR Mix (GeneCopoeia, USA), and TGX Stain-Free™ FastCast™ Acrylamide Kit (12%, Bio-Rad, USA).

Instruments comprised sterile culture dishes, CO incubators, inverted fluorescence microscopes, Transwell co-culture plates, SW-CJ-1D clean benches, high-speed centrifuges, DT5-2 low-speed centrifuges, and real-time quantitative PCR systems.

1.2 Experimental Animals and Groups Three-day-old healthy Large White piglets were obtained from Tianjin Wuqing Nongkang Pig Farm for primary isolation of porcine skeletal muscle satellite cells and intramuscular preadipocytes. The experimental design included two groups: a control group (intramuscular preadipocytes cultured alone) and a treatment group (indirect co-culture of skeletal muscle satellite cells with intramuscular preadipocytes), with four replicates per group.

1.3 Experimental Procedures 1.3.1 Isolation and Culture of Porcine Skeletal Muscle Satellite Cells and Intramuscular Preadipocytes

Under aseptic conditions, subcutaneous *longissimus dorsi* muscle was dissected

from 3-day-old piglets. Connective tissue and blood vessels were removed, and the muscle was minced into 1-2 mm^3 fragments. The tissue was digested with 0.2% type II collagenase at 37°C for 2 hours, filtered through a 200-mesh sieve, and centrifuged at 1,500 rpm for 5 minutes. After discarding the supernatant, the pellet was washed with DMEM/F12 medium, filtered through a 400-mesh sieve, and centrifuged at 1,500 rpm for 10 minutes. The resulting pellet was resuspended in DMEM/F12 complete medium containing 10% fetal bovine serum and 1% penicillin-streptomycin, then seeded into 10 cm sterile culture dishes and incubated at 37°C with 5% CO . Preadipocytes were purified using differential adhesion methods: after 2 hours of culture, the medium was removed and adherent cells were washed twice with PBS to obtain purified intramuscular preadipocytes [9]. Skeletal muscle satellite cell isolation followed the methods described by Yang et al. [10] and Yan et al. [11]. Both cell types were seeded into separate 10 cm sterile dishes and cultured at 37°C with 5% CO , with medium changes every 2 days. Cell numbers were determined using a hemocytometer.

1.3.2 Identification of Porcine Skeletal Muscle Satellite Cells and Intramuscular Preadipocytes

For skeletal muscle satellite cell identification, sterile coverslips were placed in culture dishes for cell climbing. When cells reached 70-80% confluence, differentiation medium (2% horse serum + 1% penicillin-streptomycin + DMEM/F12) was applied for 3 days. After removing the differentiation medium and washing three times with PBS, cells were fixed with acetone at room temperature for 20 minutes, washed three times with PBS, treated with Triton-X 100 (1:1,000) for 15 minutes, and washed again three times with PBS. Following blocking with bovine serum albumin (BSA) for 2 hours, Desmin antibody (1:50) was added and incubated overnight at 4°C. After three PBS washes, fluorescently labeled secondary antibody (1:2,000) was incubated in the dark for 1 hour. After three final PBS washes, nuclei were stained with 1 g/mL 4' ,6-diamidino-2-phenylindole (DAPI) for 10 minutes (protected from light), washed three times with PBS, mounted, and observed under a fluorescence microscope.

For intramuscular preadipocyte identification, cells were grown to full confluence and contact-inhibited for 2 days, then induced with complete medium containing 10 g/mL insulin for 2 days. The medium was replaced with complete medium for an additional 4 days of culture. When numerous lipid droplet-like round cells were observed under an inverted microscope, Oil Red O staining was performed to confirm intracellular lipid accumulation, verifying the cells as intramuscular preadipocytes [12].

1.3.3 Co-culture of Skeletal Muscle Satellite Cells and Intramuscular Preadipocytes

When both primary cell cultures reached 80-90% confluence, they were digested with 0.25% trypsin at 37°C, resuspended in DMEM/F12 medium, counted, and seeded into Transwell co-culture plates. Intramuscular preadipocytes were seeded in the upper chamber at 2.0×10 cells/cm^2, while skeletal muscle satel-

lite cells were seeded in the lower chamber at 1.0×10 cells/cm², using complete medium containing 4% fetal bovine serum. When skeletal muscle satellite cells reached 80% confluence, the medium was replaced with DMEM/F12 containing 2% horse serum to induce differentiation, with medium changes every 48 hours. After intramuscular preadipocytes were contact-inhibited for 2 days, differentiation was induced with complete medium containing 10 g/mL insulin for 2 days, followed by complete medium until visible lipid droplets appeared.

1.3.4 Real-time Quantitative PCR

Total RNA was extracted using the TaKaRa RNA kit according to the manufacturer' s protocol. First-strand cDNA synthesis was performed using a kit from Fermentas (USA). Gene-specific primers were as follows: PPAR (5 -ACCACTCGCATTCCTTTGAC-3 , 5 -CCACAGACTCGGCACTCAAT-3), C/EBP (5 -ATGGAGCAAGCCA ACTTCTAC-3 , 5 -GCCAGGAACTCGTCGTTGAA-3), ACC (5 -CTCCTAACTGCTGAGCTGTCTCTCT-3 , 5 -AGTCTTTCTCTTCAATTCTTGCCT-3), FAS (5 -AAGGAGGAGTCAACGGG-3 , 5 -GATGGTGAGGAGTCGGAT-3), LPL (5 -CGAAGTATTGGCATCCAGAAAC-3 , 5 -TTGATCTCATAGCCCAAGTTGTT-3), and -actin (5 -ACCACAGCCGAGAGAGAAAT-3 , 5 -GACCTGACCATCAGGGAGTT-3). All primers were synthesized by Shanghai Bioengineering Technology Service. PCR amplification conditions were: 95°C for 5 minutes, followed by 30 cycles of 94°C for 60 seconds, 55-60°C for 40 seconds, and 72°C for 40 seconds, with a final extension at 72°C for 10 minutes and hold at 4°C. Melting curves were generated, and relative quantification was performed using the 2^(-ΔΔCt) method.

1.3.5 Western Blot Analysis

Cultured cells were lysed on ice with lysis buffer and centrifuged at 10,000×g for 10 minutes at 4°C. Protein concentrations were determined in the supernatant. Total proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes. Membranes were incubated with primary antibodies against -actin, C/EBP , PPAR , FAS, and ACC (Abcam, UK), followed by blocking with 5% skim milk for 1 hour. After incubation with horseradish peroxidase-conjugated secondary antibodies at 37°C for 1 hour, protein bands were detected using ChemiDoc XRS (Millipore, USA) and Bio-Rad gel imaging system. Gray values were analyzed for relative quantification.

1.4 Statistical Analysis Data were analyzed using SPSS 17.0 software. Significant differences were determined by one-way ANOVA, with $P<0.05$ considered statistically significant and $P<0.01$ considered highly significant. Results are expressed as mean ± standard deviation (SD).

Results

2.1 Cell Morphology and Identification 2.1.1 Morphology and Identification of Skeletal Muscle Satellite Cells

Under an inverted microscope, primary satellite cells initially appeared spindle- or fusiform-shaped after attachment (Figure 1 [Figure 1: see original paper]-A). As cells proliferated and fused, they became aligned in an orderly fashion (Figure 1-B). After 2 days of differentiation, multinucleated myotubes formed with centrally located nuclei, appearing as long, parallel structures (Figure 1-C). Immunofluorescence identification using the specific porcine skeletal muscle satellite cell marker Desmin revealed positive cytoplasmic expression (Figure 1-D), confirming the cultured cells as porcine skeletal muscle satellite cells (Figure 1-E, Figure 1-F).

2.1.2 Morphology and Identification of Intramuscular Preadipocytes

Intramuscular preadipocytes grew as adherent cells. After reaching confluence and undergoing 2 days of contact inhibition (Figure 2 [Figure 2: see original paper]-A), differentiation was induced with induction medium I for 48 hours, causing cells to gradually become rounded (Figure 2-B). Subsequent treatment with induction medium II for 48 hours, followed by 4 days in complete medium containing 10% fetal bovine serum, resulted in numerous lipid droplet-like structures. Oil Red O staining confirmed abundant intracellular lipid accumulation (Figure 2-C), verifying the cells as intramuscular preadipocytes.

2.2 Effects of Skeletal Muscle Satellite Cells on Lipid Deposition in Intramuscular Preadipocytes On day 8 of intramuscular preadipocyte differentiation, numerous lipid droplets were observed. Oil Red O staining revealed abundant, large lipid droplets in control cells (Figure 3 [Figure 3: see original paper]-A), whereas co-cultured cells showed fewer lipid droplets with lighter, duller staining (Figure 3-B). Quantitative analysis demonstrated that both lipid droplet number and area were significantly lower in the co-culture group compared with the control group ($P<0.01$) (Figure 3-C, Figure 3-D). These results indicate that co-culture with skeletal muscle satellite cells inhibits intramuscular preadipocyte differentiation and lipid deposition.

2.3 Effects of Skeletal Muscle Satellite Cells on Expression of Proliferation and Differentiation Transcription Factors To verify the influence of skeletal muscle satellite cells on preadipocyte proliferation and differentiation, we examined the expression levels of C/EBP and PPAR . As shown in Figure 4 [Figure 4: see original paper], both gene and protein expression levels of PPAR and C/EBP in co-cultured intramuscular preadipocytes were significantly lower than in the control group ($P<0.01$). These findings demonstrate that skeletal muscle satellite cells suppress the expression of adipogenic marker genes in intramuscular preadipocytes.

2.4 Effects of Skeletal Muscle Satellite Cells on Expression of Key Lipid Metabolism Enzymes To further investigate the impact of porcine skeletal muscle satellite cells on lipid metabolism in intramuscular preadipocytes, we measured the expression levels of FAS, ACC, and LPL. As illustrated in Figure 5 [Figure 5: see original paper], the co-culture group exhibited significantly reduced gene and protein expression of FAS and ACC ($P<0.01$), while LPL expression was significantly increased ($P<0.01$). These results suggest that skeletal muscle satellite cells inhibit lipid anabolism while promoting lipolysis in intramuscular preadipocytes.

Discussion

Co-culture methods for muscle and adipocytes have been widely employed to study cellular growth characteristics and secretory functions. In vitro co-culture of skeletal muscle satellite cells and intramuscular preadipocytes provides an important model for investigating interactions between muscle and adipose tissues. Ailhaud et al. [13] reported that myocytes and preadipocytes maintain close contact during embryonic development. Hausman et al. [6] first attempted direct co-culture of intramuscular preadipocytes and skeletal muscle satellite cells. Although this approach approximated the in vivo environment, it yielded few adipocytes and was contaminated with other cell types, making it unsuitable for quantitative and qualitative analyses. Dodson et al. [3-4] demonstrated that co-culture of 3T3-L1 adipocytes and muscle cells promoted adipocyte survival, growth, and proliferation while inhibiting differentiation. Yan et al. [11] examined direct co-culture of porcine preadipocytes and muscle satellite cells, finding that the co-culture system enhanced adipocyte growth and proliferation while suppressing differentiation.

Our study investigated the differentiation characteristics of intramuscular preadipocytes in an indirect co-culture system with porcine skeletal muscle satellite cells. Compared with monoculture, co-cultured intramuscular preadipocytes showed reduced lipid droplet number and area, with quantitative analysis confirming highly significant differences ($P<0.01$). These findings indicate that muscle cells inhibit intramuscular preadipocyte differentiation and lipid deposition, consistent with the results of Yan et al. [11].

Previous studies have shown that FAS is a key enzyme converting acetyl-CoA and malonyl-CoA to triglycerides, regulating long-chain fatty acid synthesis in mammals. ACC is a critical enzyme in lipid metabolism, while LPL plays an essential role in triglyceride catabolism [14]. PPAR and C/EBP are highly expressed in adipose tissue and play pivotal roles in adipogenesis and lipid storage [8,15-16]. Our study revealed that co-culture significantly reduced the gene and protein expression of PPAR and C/EBP in porcine intramuscular preadipocytes. Additionally, FAS and ACC expression was markedly decreased, whereas LPL expression was significantly increased. These results demonstrate

that skeletal muscle satellite cells in the co-culture system suppress intramuscular preadipocyte differentiation while promoting lipolysis, confirming the important regulatory role of muscle cells in adipocyte development [17-18].

Conclusion

This study successfully isolated and cultured porcine skeletal muscle satellite cells and intramuscular preadipocytes, establishing an indirect co-culture system. The results confirmed that skeletal muscle satellite cells inhibit lipid deposition in intramuscular preadipocytes within this co-culture system.

References

[1] KOKTA T, DODSON M V, GERTLER A, et al. Intercellular signaling between adipose tissue and muscle tissue[J]. Domestic Animal Endocrinology, 2004, 27(4): 303–331.

[2] QUINN L S, STRAIT-BODEY L, ANDERSON B G, et al. Interleukin-15 stimulates adiponectin secretion by 3T3-L1 adipocytes: evidence for a skeletal muscle-to-fat signaling pathway[J]. Cell Biology International, 2005, 29(6): 449–457.

[3] DODSON M V, HOSSNER K L, VIERCK J L. Intercellular communication between muscle cells and 3T3-L1 preadipocytes[J]. Basic & Applied Myology Bam, 1996, 6(6): 503–512.

[4] DODSON M V, VIERCK J L, HOSSNER K L, et al. The development and utility of a defined muscle and fat co-culture system[J]. Tissue & Cell, 1997, 29(5): 517–524.

[5] DIETZE D, KOENEN M, RÖHRIG K, et al. Impairment of insulin signaling in human skeletal muscle cells by co-culture with human adipocytes[J]. Diabetes, 2002, 51(8): 2369–2376.

[6] HAUSMAN G J, POULOS S P. A method to establish co-cultures of myotubes and preadipocytes from collagenase digested neonatal pig semitendinosus muscles[J]. Journal of Animal Science, 2005, 83(5): 1010–1016.

[7] SASAO N, HIRAYAMA E, KIM J. Formation and characterization of spontaneously formed heterokaryons between quail myoblasts and 3T3-L1 preadipocytes: correlation between differential plasticity and degree of differentiation[J]. European Journal of Cell Biology, 2004, 83(1): 35–45.

[8] CHOI S H, CHUNG K Y, JOHNSON B J, et al. Co-culture of bovine muscle satellite cells with preadipocytes increases PPAR and C/EBP gene expression in differentiated myoblasts and increases GPR43 gene expression in adipocytes[J]. Journal of Nutritional Biochemistry, 2013, 24(3): 539–543.

[9] CHEN Y, ZHANG G H, LU H N, et al. Isolation, culture and induced differentiation of intramuscular preadipocytes from Bamei pigs[J]. Guangdong Agricultural Sciences, 2014, 41(13): 110-113, 123.

[10] YANG J J, LIY H, WANG K F, et al. Isolation, culture and biological characteristics of multipotent porcine skeletal muscle satellite cells[J]. Cell and Tissue Banking, 2017, 18(4): 513-525.

[11] YAN J, GAN L, YANG H L, et al. The proliferation and differentiation characteristics of co-cultured porcine preadipocytes and muscle satellite cells in vitro[J]. Molecular Biology Reports, 2013, 40(4): 3197-3202.

[12] SUN C, QI R L, WANG L, et al. p38 MAPK regulates calcium signal-mediated lipid accumulation through changing VDR expression in primary preadipocytes of mice[J]. Molecular Biology Reports, 2012, 39(3): 3179-3184.

[13] AILHAUD G, P GRIMALDI A, NÉGREL R. Cellular and molecular aspects of adipose tissue development[J]. Annual Review of Nutrition, 1992, 12(1): 207-233.

[14] LI X Z, YAN C G, YU J, et al. Dietary whole and cracked linseed increases the proportion of oleic and -linolenic acids in adipose tissues and decreases stearoyl-coenzyme A desaturase, acetyl-coenzyme A carboxylase, and fatty acid synthase gene expression in the longissimus thoracis muscle of Yanbian yellow cattle[J]. Journal of Animal Science, 2017, 95(2): 718-726.

[15] FERRAND N, BÉREZIAT V, MOLDES M, et al. WISP1/CCN4 inhibits adipocyte differentiation through repression of PPAR activity[J]. Scientific Reports, 2017, 7: 1749.

[16] CHOI S K, PARK S, JANG S B, et al. Cascade regulation of PPAR and C/EBP signaling pathways by celastrol impairs adipocyte differentiation and stimulates lipolysis in 3T3-L1 adipocytes[J]. Metabolism, 2016, 65(5): 646-654.

[17] PARK S, BAEK K, CHOI C. Suppression of adipogenic differentiation by muscle cell-induced decrease in genes related to lipogenesis in muscle and fat co-culture system[J]. Cell Biology International, 2013, 37(9): 1003-1009.

[18] HAN H Y, WEI W, CHU W W, et al. Muscle conditional medium reduces intramuscular adipocyte differentiation and lipid accumulation through regulating insulin signaling[J]. International Journal of Molecular Sciences, 2017, 18(8): 1799.

Note: Figure translations are in progress. See original paper for figures.