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Effects of *MARCKS* gene on skeletal muscle growth and development in chicken and conditional knockout mice

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ABSTRACT: The *MARCKS* gene, encoding a protein crucial for various cellular processes, was initially discovered as a substrate for protein kinase C, a family of serine/threonine kinases involved in cell proliferation, differentiation, and apoptosis. The function of this gene in chicken skeletal muscle growth and development is limitedly explored. This study investigates the effects of *MARCKS* gene on proliferation, differentiation, and apoptosis of primary chicken myoblasts, followed by analyzing its effects on skeletal muscle growth and development *in vivo* through conditional knockout mice. Overexpression of *MARCKS* significantly downregulated *MyoD1* and *MyoG* gene expression, indicating its inhibitory role in myoblasts proliferation and differentiation. At the individual level, conditional *MARCKS* knockout promoted muscle strength in mice and increased expression of *MyoD1* gene in multiple skeletal tissues. These results contribute to further understanding the function of the *MARCKS* gene in skeletal muscle.

KEYWORDS: skeletal muscle, chicken, *MARCKS*, cell proliferation and apoptosis, conditional knockout mice

INTRODUCTION

The *MARCKS* gene, Myristoylated Alanine-Rich Protein Kinase C Substrate, is a highly conserved gene encoding a protein involved in diverse cellular processes such as cell adhesion, migration, and membrane trafficking [1–3]. It was first identified and characterized in the early 1980s [2, 4]. The protein encoded by this gene is ubiquitously expressed in various tissues and has been implicated in multiple physiological and pathological conditions [5, 6]. *MARCKS* is a membrane-associated protein that is widely present in eukaryotic cells. It was initially identified as the main substrate of protein kinase C (PKC). PKC plays a significant role in neurological diseases, cardiovascular diseases, and metabolic disorders, such as myocardial contraction, electrical conduction, and vascular tension regulation [7], as well as Alzheimer's disease [8]. The *MARCKS* protein is unique, containing a myristoylation site enabling association with cellular membranes [4, 9, 10]. This myristoylation is crucial for function and localization within the cell. The primary structure of the *MARCKS* protein consists of three domains: an N-terminal myristoylated domain, a central effector domain (ED), and a C-terminal domain [11]. The ED contains phosphorylation sites for PKC and other kinases, crucial in regulating the function of the *MARCKS* protein [12]. The C-terminal domain is involved in protein-protein interactions, binding to several partners, including actin and calmodulin [13, 14].

The *MARCKS* protein plays a pivotal role in regulating the actin cytoskeleton, a critical aspect for cellular functions like cell migration and adhesion [4]. Its direct interaction with F-actin through its ED influences actin filament dynamics [15]. Phosphorylation by PKC further modulates the actin-binding activity of the *MARCKS* protein, offering a mechanism for dynamic actin cytoskeleton regulation [16]. Beyond its involvement in the actin cytoskeleton, the *MARCKS* protein contributes to diverse cellular processes. It participates in membrane trafficking events, including endocytosis and exocytosis [17]. Additionally, the *MARCKS* protein regulates various ion channels, such as voltage-gated potassium channels and NMDA receptors [18, 19]. Recent studies have unveiled novel roles in cell signaling pathways like the phosphoinositide 3-kinase (PI3K)/Akt pathway [20].

Moreover, the *MARCKS* gene plays a crucial role in neuronal development and function as well as in cancer [21]. Dysregulation of the *MARCKS* gene or its protein product has been associated with neurological disorders, including Alzheimer's disease and schizophrenia [22], and is also implicated in the development and progression of cancers such as breast, lung, and prostate cancer [23–25]. Therefore, targeting the *MARCKS* protein or its associated signaling

pathways has emerged as a promising therapeutic strategy for treating these diseases [26, 27].

Skeletal muscle development, also known as *Myo*Genesis, is an intricate and highly regulated process involving the proliferation and differentiation of myoblasts into myotubes, ultimately forming mature muscle fibers [28]. The *MARCKS* gene is implicated in various stages of *Myo*Genesis, particularly during the early phases of myoblast differentiation. Studies demonstrate that *MARCKS* expression is upregulated during *Myo*Genic differentiation, indicating its crucial role in this process [29]. The primary function of the *MARCKS* protein is its capacity to bind and cross-link actin filaments, thereby regulating actin cytoskeleton remodeling [4]. This regulation is pivotal for stabilizing the cytoskeletal structure. Furthermore, *MARCKS* acts as a substrate for PKC alpha, potentially influencing PKC activity. Interactions and co-localization between PKC isozymes and *MARCKS* at focal adhesion sites are also observed [30]. The interaction between *MARCKS* and PKC is essential for muscle development. *MARCKS* serves as a significant, PKC-releasable reservoir of calmodulin (CaM) in differentiated smooth muscle, contributing to CaM signaling by modulating the intracellular distribution of CaM [31].

MARCKS emerges as a novel translational target with cell type-specific effects on both Endothelial Cells (ECs) and Vascular Smooth Muscle Cells (VSMCs). Knockdown of *MARCKS* results in decreased KIS expression, leading to nuclear trapping of p27kip1 in VSMCs, thereby arresting the cell cycle [32]. During *Myo*Genesis, the phosphorylation pattern of many proteins in chick embryonic muscle cells undergoes significant changes. *MARCKS*, a major substrate of PKC, is identified as a 63 kDa protein with declining phosphorylation during differentiation. The decrease in *MARCKS* phosphorylation is attributed to a reduction in its cytosolic level and an increase in its membrane fraction. *MARCKS* translocates from the cytosol to the plasma membrane and the peripheral region of nuclei as myoblasts fuse to form multinucleated myotubes. The phosphorylation of *MARCKS* is controlled by PKC-theta, and its downregulation during *Myo*Genesis contributes to *MARCKS* translocation, which is associated with or required for myoblast fusion [33].

Chicken skeletal muscle development encompasses prenatal *Myo*Genesis and postnatal hypertrophy of myofibers, orchestrated by a dynamic interplay between gene regulatory networks and genome topology [34]. The limited understanding of *MARCKS*' mechanisms in skeletal muscle development prompted us to investigate the intricate regulatory orchestration of gene expression in this context. To enhance the understanding of *MARCKS* functions, we systematically delineated its regulation profiles using conditional knockout mice. Notably, there is a 51.47% sequence similarity for *MARCKS* protein between chickens and mice.

MATERIALS AND METHODS

Ethical statement

All animal experiments adhered to the Guidelines for Experimental Animals established by the Ministry of Science and Technology (Beijing, China, revised in March 2017). The Ethics Committee of Chengdu University granted ethical approval for this study (protocol number 2021202024).

Animals

To obtain primary myoblasts, we followed standard procedures to collect 100 Qingjiao chicken eggs from DEKON GROUP's farm (DEKON, Chengdu, China), located in the Autonomous Prefecture (temperature 37.8 °C, moderate humidity 60%). C57/BL6J mice were procured from GemPharmatech Co., Ltd. (Nanjing, China), the Company also provided *MARCKS* conditional knock mice. Wild type (WT) and conditional knockout mice (CKO) required for subsequent experiments were obtained through selective breeding. Genotype identification relied on specific primers (Table S1).

Histological analysis

Tissue samples underwent fixation for 12–24 h in 4% neutral buffered formalin. Subsequent steps included paraffin embedding, sectioning, and staining with hematoxylin and eosin for subsequent image analysis. Within each sample image, we identified and measured the diameter and cross-sectional area of 200 clearly delineated muscle fibers. To address potential bias from incomplete muscle fiber transection, measurements were specifically taken from locations with shorter diameters. Additionally, we excluded 50 measurements each from the largest and smallest values before conducting statistical analyses on both diameter and cross-sectional area. Furthermore, we tallied the number of muscle cells in 10 muscle bundles and performed multiple comparisons to highlight differences between conditional KO and WT mice.

Cell culture

On the 10th day of incubation, primary myoblasts were isolated from chicken leg muscle. Following bone removal, the leg muscle was immersed in DMEM (Gibco, USA) medium supplemented with 20% Fetal Bovine Serum (Procell, Wuhan, China) and 0.1% penicillin/streptomycin (Gibco). The muscle was dissected, placed in a 50 ml centrifuge tube, and the suspension was gently swirled for 1 min. After filtration using a 100 μm cell sieve, the filtrate was centrifuged at 1,000 rpm for 5 min at room temperature, and the resulting cells were collected. These cells were re-suspended in a growth medium, transferred to a culture bottle, and cultured in a 5% CO_2 environment at 37 °C. The cells underwent differential adhesion, a process repeated three times. Daily observations and

recording of cell growth were conducted throughout the experiment.

DNA extraction, RNA extraction, cDNA synthesis, and quantitative real-time fluorescence PCR (qRT-f-PCR)

DNA extraction from the toes of one-week-old mice used the Blood/Cell/Tissue Genomic DNA Extraction Kit (Tiangen, Beijing, China) for genotype identification. DNA concentration was determined using a spectrophotometer. PCR assays were conducted using Vazyme 2 × Rapid Taq Master Mix (Vazyme, Nanjing, China), and mouse genotypes were identified through electrophoresis on a 1% agarose gel. RNA extraction from tissues and cells was performed using RNAiso Plus (Takara, Beijing, China), and RNA concentration was determined with a spectrophotometer. The cDNA chains were synthesized using the ExonScript RT SuperMix with dsDNase reverse transcription kit (Rongwei, Chengdu, China).

The LightCycler96 Instrument system quantified samples using chick β-actin gene and mouse GAPDH gene as the internal references. We conducted qRT-PCR with the Fast SYBR Green qPCR Master Mix UDG kit (Rongwei). All experiments occurred in triplicate, and we employed the $2^{-\Delta\Delta Ct}$ method for relative quantification. Primers for these experiments were synthesized by Shanghai Sangon Bioengineering Co., Ltd. (Chengdu, China), and their sequences are provided in Table S2.

Construction of vector

We developed overexpression vectors (PEGFP-*MARCKS*) for *MARCKS* transcripts (NM_205480.1) with the assistance of Beijing Tsingke Biotech Co., Ltd. (Beijing, China). Additionally, three siRNA sequences (*MARCKS*-174, *MARCKS*-265, *MARCKS*-355) crucial for the gene interference test were designed and synthesized by Shanghai Sangon Bioengineering Co., Ltd. (Shanghai, China). Specific details are outlined in Table S3.

Overexpression and interference efficiency assays

Upon achieving approximately 80% confluence in a T175 culture flask, cells were digested, counted, and transferred to 24-well plates. Transfection with overexpression and interference vectors occurred at a cell confluence of 40% to 50%, with mock siRNA or mock expression vectors as negative controls. The culture medium was changed 8 h post-transfection. Cell RNA samples were collected at 12 h, 24 h, and 48 h after transfection for qRT-PCR detection of the target gene *MARCKS* and the internal reference gene β-actin. Overexpression and interference vector transfection efficiency were analyzed to identify the optimal time point for transfection.

Immunofluorescence

Myoblast purity was assessed through a desmin immunofluorescence assay utilizing a desmin antibody and a rabbit IgG FITC-conjugated antibody. Cell differentiation was detected using *MYHC* and FITC-conjugated anti-mouse IgG antibodies (Servicebio, Wuhan China), with nuclear staining achieved with DAPI. Image acquisition occurred using a fluorescence microscope.

Body weight analysis and growth curve determination

Mice's body weight was documented weekly from birth to the 8th week, categorizing the data into WT and CKO groups based on genotype identification. Collected data underwent analysis using GraphPad Prism 9 to generate a growth curve for the mice.

Four-claw suspension test

To conduct the four-claw suspension test, we prepared a square of barbed wire secured at a height of approximately 40 cm from the ground. The tail of the mouse was raised and placed in the center of the grid, and when the mouse successfully grasped the grid with all four claws, the tail was released, then the grid was inverted. A plastic basket was positioned beneath the barbed wire, and filled with an appropriate amount of bedding material to prevent mice from falling and sustaining injuries during the test. Timing began when the mouse was suspended upside down on the wire mesh and concluded when the mouse fell from the wire mesh. The time taken by each mouse was recorded.

Swimming endurance test

Prior to initiating the test, in order to shorten the experiment time, we documented the weights of the mice and determined the necessary load weight as 5% of each mouse's body weight. Small iron blocks were prepared accordingly and secured to the tails of the mice using thin wire. The mice were then placed in a thermostatic pool with the water temperature maintained at (25.0 ± 0.5) °C, and the water depth was kept at a minimum of 30 cm. Timing commenced as the mice were introduced into the water, with continuous agitation to ensure movement of their limbs. If a mouse failed to surface within 3 to 5 s while submerged, the time was noted. Subsequently, the mice were promptly removed, gently dried with a towel, and placed in a warm environment for recovery.

Statistical analysis

In this study, we primarily utilized Primer 5.0, BioRad CFX, SPSS 8.0, GraphPad Prism 9, Image Plus, and other software. The experimental data from fluorescence qPCR were processed using the $2^{-\Delta\Delta Ct}$ method and plotted using GraphPad Prism 9.0. Results were expressed as Mean ± standard error (SEM). When $p >$

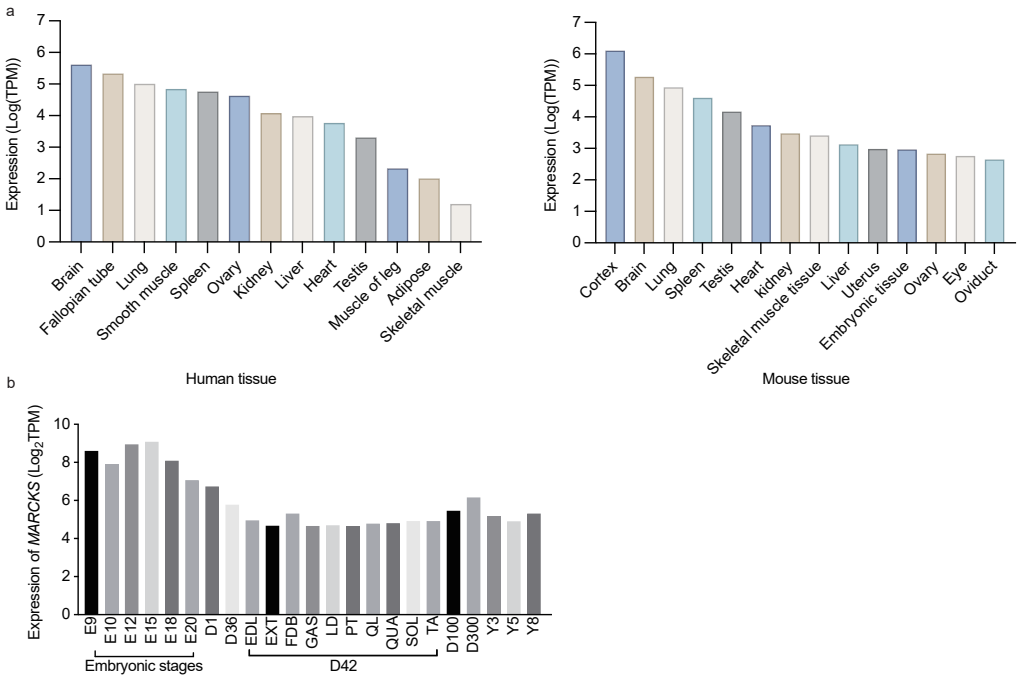

Fig. 1 *MARCKS* gene expression of different human and mouse tissues. (a) Expression of the *MARCKS* gene expression of 10 different muscle tissues in White-feathered broiler and at different time points in chicken skeletal muscle. (b) E9–E20: Embryo Day 9 to Embryo Day 20; D1, Day 1 after birth; D42, Day 42 after birth; EDL, Extensor digitorum longus; EXT, Extraocular; FDB, flexor digitorum brevis; GAS, Gastrocnemius; LD, Longissimus dorsi; PT, Pectoralis thoracica; QL, Quadratus lumbrorum; QUA, Quadriceps; SOL, Soleus; TA, Tibialis anterior; D100, Day 100 after birth; D300, Day 300 after birth; Y3, 3 years old; Y5, 5 years old; Y8, 8 years old.

0.05, the difference was not significant; when $0.01 < p < 0.05$, it indicates a significant difference, represented by '*' in the figure; when $p < 0.01$, it indicates an extremely significant difference, represented by '**' in the figure. Each set of data involved at least three biological replicates.

RESULTS

MARCKS expression across development stages and anatomical positions of skeletal muscle tissue

We obtained the expression profiles of *MARCKS* from https://www.ebi.ac.uk/gxa/home. The *MARCKS* mRNA exhibited widespread expression across different tissues in both human and mouse, with notably higher expression in the brain and cortex (Fig. 1a), indicating the gene's extensive functionality. Considering the limited investigation of this gene in chicken muscle development, we downloaded transcriptome data from 58 skeletal muscle samples of 42-day-old White-feathered broiler chickens (PRJNA837345) [35], and 32 leg skeletal muscle samples across 12 developmental stages of Tibetan chicken (PRJNA866681). Additionally, 6 skeletal muscle samples at the embryonic age of 10 days old (PRJNA1066518) were included for integrated analysis. Interestingly, we observed that the *MARCKS* gene exhibited time-dependent expression, with higher expression during the embryonic stage (Fig. 1b). Subsequently, we utilized KO mice to explore the detailed regulatory functions of *MARCKS* in muscle development.

Inhibition of myoblast proliferation by *MARCKS*

Upon obtaining chicken primary myoblasts, we identified them through desmin immunofluorescence (Fig. 2a). To modulate the mRNA expression level of the *MARCKS* gene in chicken primary myoblasts, we constructed and synthesized siRNA (*MARCKS*-174, *MARCKS*-265, and *MARCKS*-355) and overexpression vectors of the *MARCKS* gene (PEGFP-*MARCKS*).

The interference efficiency of *MARCKS*-355 was found to be significant and stable at 24 h after transfection, as depicted in Fig. 2b. Transfection with 25 nmol/l *MARCKS* siRNA resulted in a substantial $73 \pm 1\%$ reduction in *MARCKS* mRNA levels after a 24 h transfection period ($p < 0.001$, Fig. 2b). Consequently, *MARCKS*-355, the siRNA with optimal interference, was selected for subsequent gene function research.

To investigate the impact of the *MARCKS* gene on the differentiation and fusion of chicken primary myoblasts, we examined genes associated with myoblast proliferation and differentiation 72 h after induction

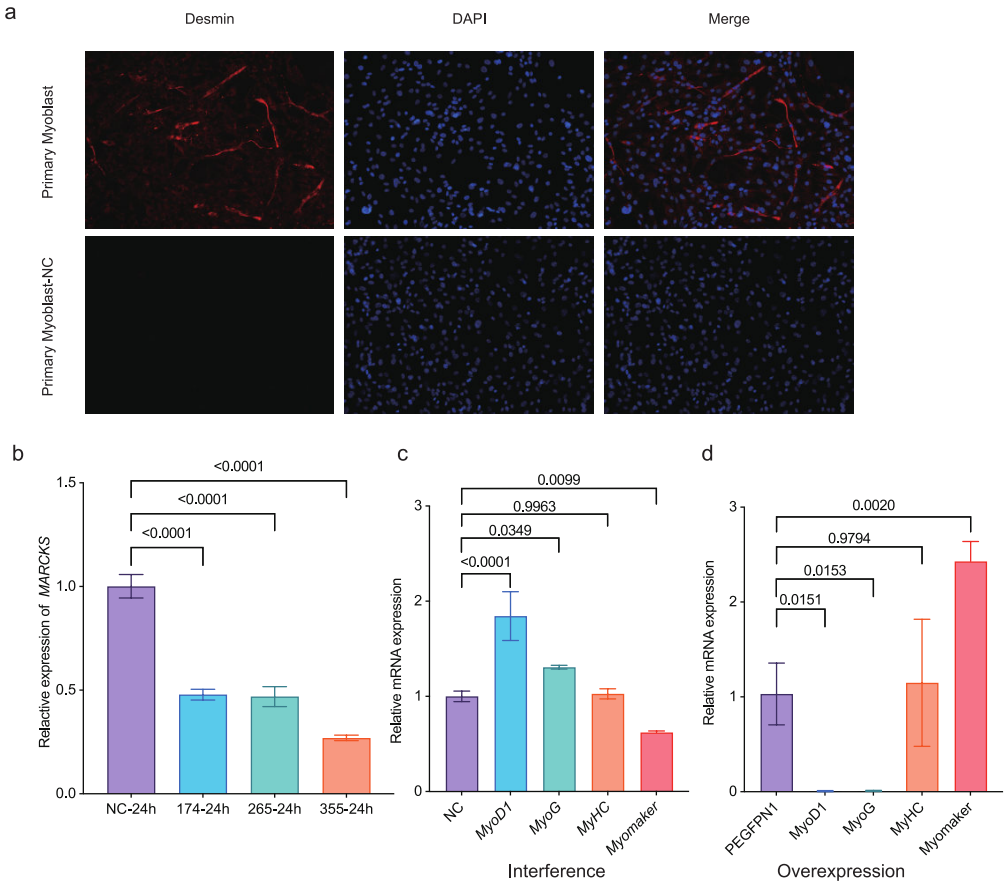

Fig. 2 Chicken primary myoblasts phenotype after interference and overexpression of the *MARCKS* gene. (a) Desmin immunofluorescence of chicken primary myoblast. (b) RT-qPCR analysis of the RNA interference efficiency of the *MARCKS* gene at 24 h. The expression of myoblast proliferation and differentiation related genes after interference (c) and expression (d). Data are presented as mean ± standard deviations (SD). ***, $p < 0.001$, via t-test. NC, transfection with control siRNA.

using qRT-PCR. In the interference groups, compared with the NC group, the expressions of *MyoD1* and *MyoG* genes significantly increased after *MARCKS*-355 transfection, while the expression of *Myomarker* was notably downregulated ($p < 0.05$). Similarly, in the overexpression groups, transfection of PEGFPN1-*MARCKS* led to a significant down-regulation in the expressions of *MyoD1* and *MyoG* genes, while the expression of *Myomarker* was significantly up-regulated ($p < 0.05$). This indicates the inhibitory role of *MARCKS* in myoblast proliferation. There was no significant change in the expression level of *MyHC* among the interference and overexpression groups (Fig. 2c,d).

Impact of *MARCKS* deletion on body weight and kinematic performance in adult mice

To elucidate the role of *MARCKS*, we conducted a CKO of this gene in mice, comparing phenotypic variations between WT and CKO counterparts. Mouse bodyweight data were meticulously recorded from the 1st to the 7th week post-birth, and weight fluctuations in mice of both genotypes were analyzed. No significant differences in body weight were observed between WT and CKO mice across all time points. A discernible trend suggested higher body weight in female CKO mice, yet statistical significance was not achieved (Fig. 3a).

The endurance of muscles in physical activities is intricately linked to muscle fiber type composition. To assess limb muscle endurance and movement abilities, we employed exhaustive swimming endurance, longest running distance, and four-paw tension tests on 8-week-old male mice. Results indicated no significant differences in exhaustive swimming endurance time and longest running distance between WT and CKO mice ($p > 0.05$), implying that *MARCKS* muscle CKO did not impact the endurance performance of mice. However, in the four-claw pull test, CKO mice exhibited significantly higher four-claw pull strength compared to the WT group (Fig. 3b), suggesting a potential increase in muscle strength due to *MARCKS* muscle CKO.

Investigating the impact of *MARCKS* gene KO on the muscle fiber morphology in the skeletal muscle

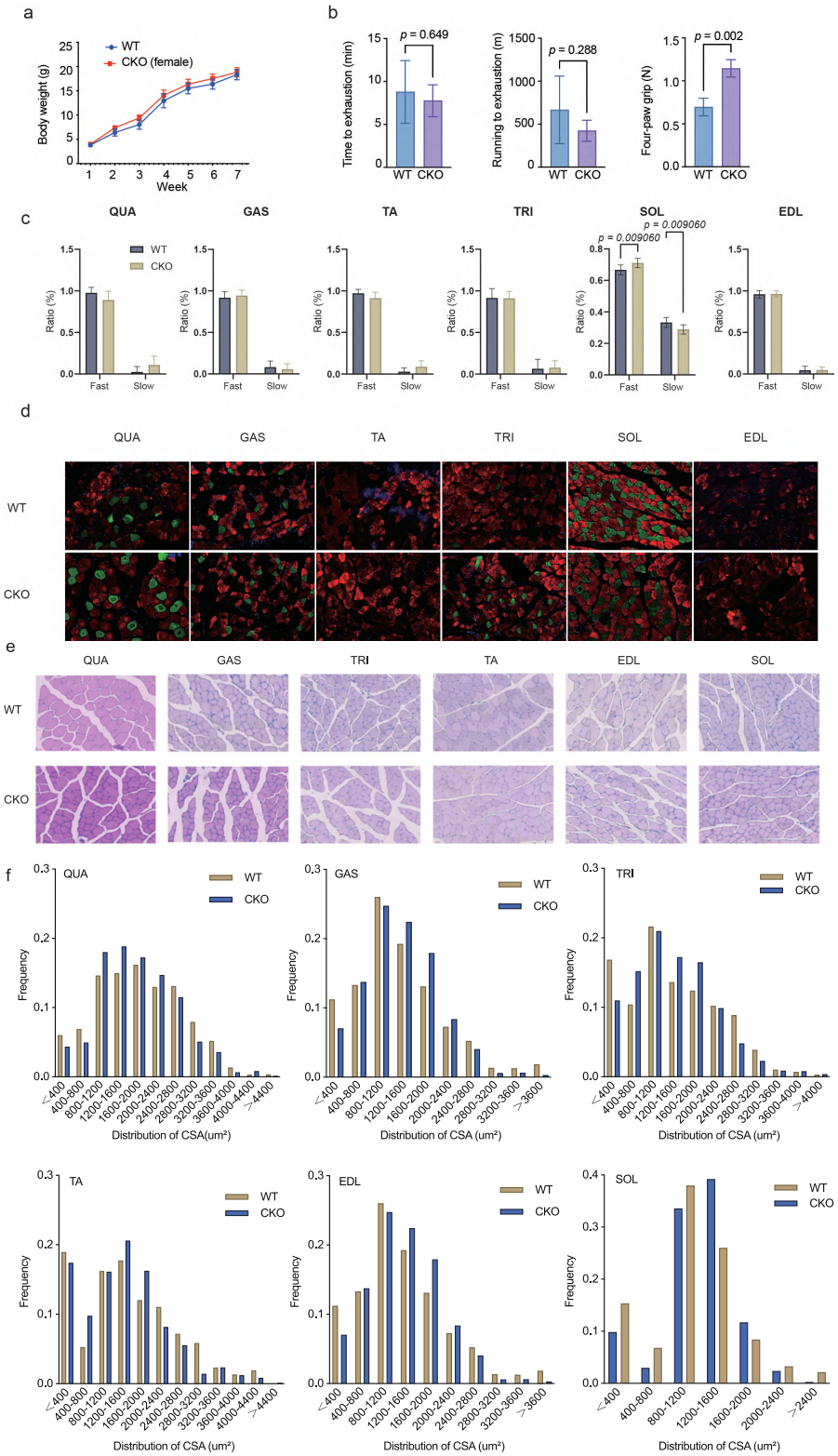

Fig. 3 Phenotypic difference between wide type and *MARCKS* CKO mice. (a) Body weight growth curves of female mice with different genotypes. (b) Exhaustive swimming test (left), running test (middle), and four-paw grip test (right) of 8-week-old male mice. Expression of *MARCKS* among different tissues. (c) Ratio of fast and slow muscle fibers in each skeletal muscle tissue. (d) 6 skeletal muscle sections were stained with fast and slow muscle immunofluorescence. (e) Histological section of skeletal muscle across 6 regions. (f) Cross-sectional area comparison of 6 skeletal muscle regions.

of mice, we conducted paraffin sections of muscle tissue from six muscle parts of 10-week-old mice after fixation and dehydration. Microscopic examination of muscle fiber characteristics revealed that the proportion of fast muscle fibers in the soleus (SOL) of CKO mice was significantly higher than that of WT mice, while the proportion of slow muscle fibers was significantly lower. In the remaining five muscle parts, no significant differences were observed in the proportion of different types of muscle fibers between WT and CKO mice (Fig. 3c,d). Cross-sectional area comparison across six skeletal muscle regions between WT and CKO indicated a decrease in myofibers with a cross-sectional area of 1200–1600 mm in SOL (Fig. 3e,f), though none of these comparisons reached statistical significance.

Impact of *MARCKS* CKO on gene expression in adult mice pertaining to skeletal muscle development

We assessed the mRNA levels of key genes—*MyoD1*, *MyoG*, *MyHC*, and *Myomaker*—in the skeletal muscle tissues of 10-week-old mice with two distinct genotypes. These genes serve as markers associated with muscle proliferation, differentiation, and fusion, with *Myomaker* playing a crucial role as a membrane activator in myoblast fusion and muscle formation [34]. Comparative analysis revealed a noticeable trend of upregulation in *Myomaker* expression within the two skeletal muscles of single-KO mice, although the difference did not reach statistical significance ($p > 0.05$) compared to WT mice. Furthermore, the expression levels of *MyoD1*, *MyHC*, and *MyoG*, linked to muscle proliferation and differentiation, exhibited minimal changes across the four muscle tissues, except in the triceps brachii (Fig. 4). These findings suggest a potential inhibitory effect of *MARCKS* on muscle fusion.

Impact of *MARCKS* CKO on the expression of muscle fiber-related genes in mouse skeletal muscle

To delve deeper into the role of *MARCKS* in influencing muscle fiber types, we assessed the number and expression levels of fast and slow muscle-related genes in six distinct muscle segments across two genotypes of mice. *MyHC* IIa, *MyHC* IIb, *MyHC* IIx, *Tnnc2*, and *Tnni2* serve as markers for fast muscle fibers, while *MyHC* I, *Tnnc1*, and *Tnni1* are indicative of slow muscle fibers.

Our findings reveal a notable upregulation in the expression of slow muscle fiber-related marker genes within the quadriceps (QUA), contrasting with an increase in the expression of fast muscle fiber-related marker genes in the gastrocnemius (GAS), digital extensor longus (EDL), and soleus (SOL) muscles of these *MARCKS* CKO mice ($p < 0.05$). Conversely, a significant decrease ($p < 0.05$) in the expression of slow muscle fiber-related marker genes was observed, accompanied by opposing results in the triceps brachii and tibialis anterior muscles (TA). These outcomes suggest a dual role for *MARCKS*: inhibiting slow muscle formation in QUA, promoting fast muscle formation while inhibiting slow muscle formation in GAS, EDL, and SOL, and influencing a converse pattern in triceps brachii and tibialis anterior muscles (TA) (Fig. 5).

DISCUSSION

The *MARCKS* gene encodes a multifunctional protein that actively regulates various cellular processes, encompassing cell adhesion, migration, secretion, proliferation, and membrane trafficking [3, 37]. Recent research has concentrated on comprehending the involvement of the *MARCKS* gene in skeletal muscle development and function. The role of *MARCKS* in skeletal muscle is interconnected with its interaction with other proteins implicated in cellular signaling. Specifically, *MARCKS* has been demonstrated to bind to and modulate the activity of PKC3. PKC is recognized for its regulatory influence on diverse aspects of muscle function, such as contraction and metabolism [38]. Thus, the interaction between *MARCKS* and PKC bears significant implications for skeletal muscle physiology.

In this study, we demonstrate that *MARCKS* is ubiquitously expressed in different parts of chicken muscles and at various stages, with notable expression during the embryonic stage, suggesting its impact on early muscle development and sustained growth. Previous research by Kim et al [29, 33] has revealed that PKC-controlled *MARCKS* translocation is a prerequisite for myoblast fusion, a pivotal cellular event shaping the formation, fusion, and repair of embryonic muscle cells. In zebrafish embryos, inhibiting the two *MARCKS* paralogs, *MARCKS* a and *MARCKS* b, led to severe phenotypic defects, including curved and truncated tails, gill-formation abnormalities, skeletal muscle deformities, and an abnormal brain architecture [39]. To delve deeper into the function of the *MARCKS* gene, siRNA and over-expression vectors of the *MARCKS* gene were constructed and synthesized to assess its impact on myoblasts. Our study reveals that overexpression with *MARCKS* significantly downregulates *MyoD1* and *MyoG* gene expression, indicating the inhibitory role of *MARCKS* in myoblast proliferation and differentiation.

In the case of *MARCKS* gene CKO mice, we observed that the CKO of the *MARCKS* gene enhances muscle strength in mice and increases the expression of the *MyoD1* gene in multiple skeletal tissues. This aligns with a previous study by Wang et al [32], which demonstrated that *MARCKS*-deficient mice exhibit reduced muscle force generation and fatigue resistance. These findings suggest that the *MARCKS* gene contributes to the functional properties of skeletal muscle fibers. Thus, by examining the expression levels of fast and slow muscle-related genes in six different muscle segments of the two genotypes of

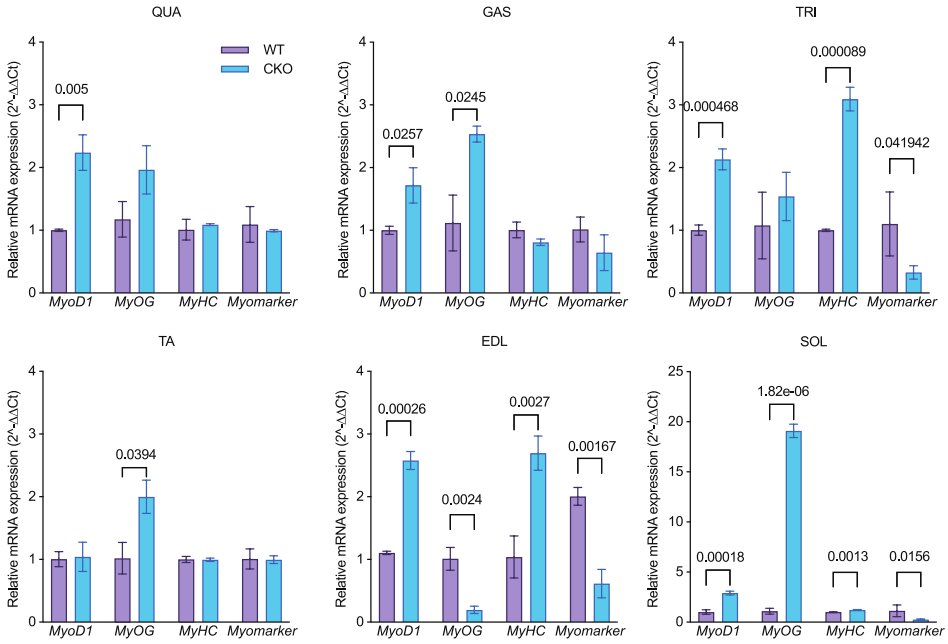

Fig. 4 Expression of skeletal muscle development related genes in six muscle tissues of mice Quadriceps (QUA), Gastrocnemius muscle (GAS), Triceps brachii (TRI), Tibial anterior muscle (TA), Extensor digitorum longus (EDL), Soleus (SOL), respectively.

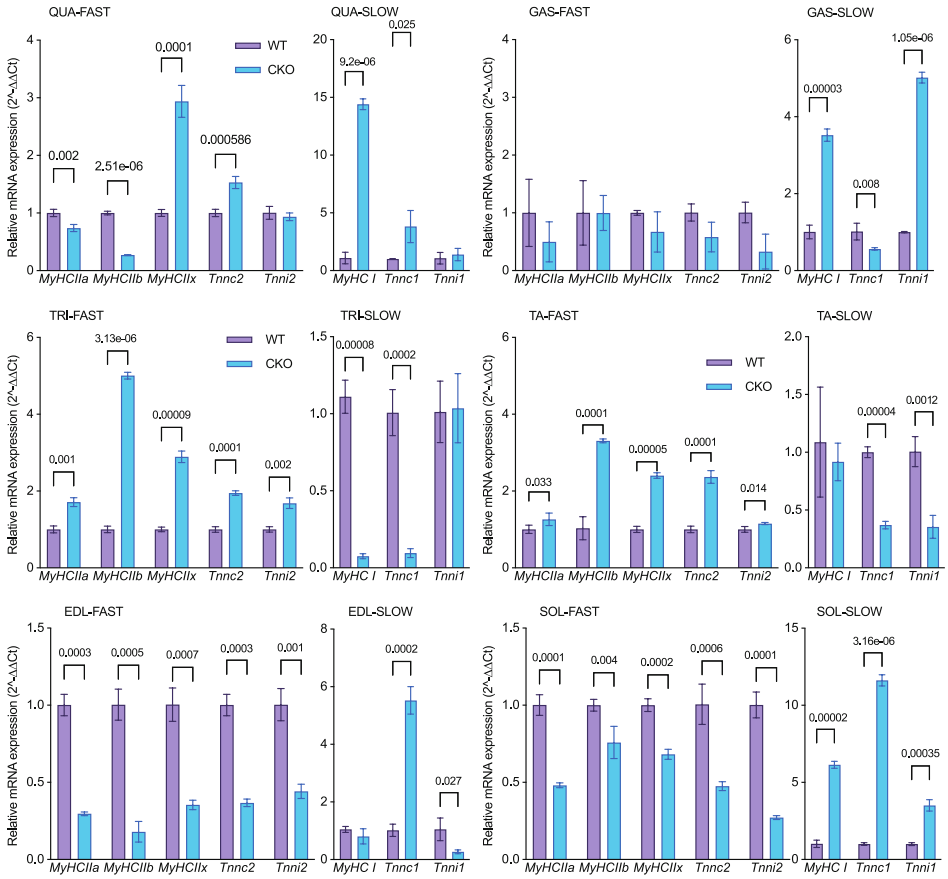

Fig. 5 Effect of *MARCKS* gene CKO on fast and slow muscle fibers. Expression of skeletal muscle development related genes in six muscle tissues of mice in Gastrocnemius muscle (GAS), Quadriceps (QUA), Tibial anterior muscle (TA), Triceps brachii (TRI), Extensor digitorum longus (EDL), and Soleus (SOL), respectively.

mice, we showed that *MARCKS* has a dual role in the development of six muscle fiber types. Our study further confirms that *MARCKS* simultaneously inhibits slow muscle formation in QUA, promotes fast muscle formation, and inhibits slow muscle formation in GAS, EDL, and SOL. Additionally, research indicates that the *MARCKS* gene may be involved in the response of skeletal muscle to exercise. Pérez-Schindler et al [40] reported that endurance exercise training increases *MARCKS* expression in mouse skeletal muscle, suggesting a potential role for the *MARCKS* gene in mediating exercise-induced adaptations in skeletal muscle. In conclusion, the *MARCKS* gene exhibits multiple effects on skeletal muscle, encompassing roles in myoblast differentiation, fusion, proliferation, and exercise response. Further research is needed to fully elucidate the molecular mechanisms underlying these effects and explore potential therapeutic applications targeting the *MARCKS* gene in skeletal muscle disorders.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2026.002.

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Appendix A. Supplementary data

Table S1 Primers used to identify mice genotype.

Primer name	Sequence	Product size
flox-F1	GACTTTTCAAATGGTATCCTGGTGTG	WT: 319 bp
flox-R1	TCTTGCCCCGGTAGGTTTAGAG	Targeted: 421 bp
flox-F2	CATCGCATTGTCTGAGTAGGTG	WT: 0 bp
flox-R2	CGAGACAGGGTTTCTCTGTATAGCC	Targeted: 334 bp
null-F	TTCCAAGTGCCTTTGCGATG	WT: 5140 bp Flox: 5345 bp
null-R	CTACTACTGCCCAGCAAGTGATTCT	Null: 432 bp
Cre-F1	CCCAGCAGGATCACATAGGCAG	WT: 0 bp
Cre-R1	AGCCCAGGATCTGCACACAGA	Targeted: 385 bp
Cre-F3	AACTTGGGCTCCTGATGTTTCCTTG	WT: 0 bp
Cre-R3	GTGCTGTTGGATGGTCTTCACAG	Targeted: 459 bp

Table S2 Primers for RT-PCR.

Gene	Primer sequence (5′ − 3′)	Product length (bp)	Tm (°C)
Gal-*MARCKS*	F:GTTTGTTGGAGTGGTGCCAGGTAC R:CGCCCGTTGGAATGGTGGTAAC	143	60 60
Gal-*MyoD1*	F:GCTACTACACGGAATCACCAAAT R:CTGGGCTCCACTGTCACTCA	200	60 60
Gal-*MyoG*	F:CGGAGGCTGAAGAAGGTGAA R:CGGTCCTCTGCCTGGTCAT	320	60 60
Gal-*MyHC*	F:CTCCTCACGCTTTGGTAA R:TGATAGTCGTATGGGTTGGT	213	60 60
Gal-*Myomarker*	F:TGGGTGTCCCTGATGGC R:CCCGATGGGTCCTGAGTAG	135	51 51
Gal-β-actin	F:TTGTTGACAATGGCTCCGGT R:AACCATCACACCCTGATGTCT	110	58 58
Mus-*MyoD1*	F:CGAGCACTACAGTTGGCGACTAAGAT R:GCTCCACTATGCTGGACAGGCAGT	204	60 60
Mus-*MyoG*	F:CCATCCAGTACATTGAGCGCCTACA R:ACGATGGACGTAAGGGAGTGCAGAT	241	60 60
Mus-*MyHC*	F:CAAGTCATCGGTGTTTGTGG R:TGTCGTACTTGGGCGGGTTC	158	60 60
Mus-*Myomarker*	F:ATCGCTACCAAGAGGCGTT R:CACAGCACAGACAAACCAGG	107	60 60
Mus-*MyHC* I	F:ACTGTCAACACTAAGAGGGTCA R:TTGGATGATTTGATCTTCCAGGG	114	60 60
Mus-*MyHC* IIa	F:AAGTGACTGTGAAAACAGAAGCA R:GCAGCCATTTGTAAGGGTTGAC	222	60 60
Mus-*MyHC* IIb	F:TTGAAAAGACGAAGCAGCGAC R:AGAGAGCGGGACTCCTTCTG	190	60 60
Mus-*MyHC* IIx	F:GCGAATCGAGGCTCAGAACAA R:GTAGTTCCGCCTTCGGTCTTG	138	60 60
Mus-*Tnni1*	F:TGAAGCCAAATGCCTCCACAACAC R:ACACCTTGTGCTTAGAGCCCAGTA	155	60 60
Mus-*Tnnc1*	F:AGCTCATGAAGGACGGTGACAAGA R:AACCGTGCAAGACCAGCATCTACT	102	60 60
Mus-*Tnni2*	F:AGCAGCAAGGAGCTGGAAGA R:ATGGCGTCGGCAGACATAC	101	60 60
Mus-*Tnnc2*	F:CCATCATCGAGGAGGTGGAC R:CTTCCCCTTCGCATCCTCTT	101	60 60
Mus-*MARCKS*	F:TGGTGCCAGGTACTGGTTTT R:AGCTGTCTAACACGTCCTCG	193	60 60
Mus-*GAPDH*	F:CATGGCCTTCCGTGTTCCTA R:CCTGCTTCACCACCTTCTTG	104	60 60

Table S3 *MARCKS* gene interference sequences and control sequences.

Fragment name	Sequence (5′ − 3′)
gga*MARCKS*-265	Sense: AGGAGGAGGUGCAGGCCAATT Antisense: UUGGCCUGCACCUCCUCCUTT
gga*MARCKS*-174	Sense: AGGCGAAUGGACAGGAAAATT Antisense: UUUUCCUGUCCAUUCGCCUTT
gga*MARCKS*-355	Sense: ACGUGAAGGUGAACGGCGATT Antisense: UCGCCGUUCACCUUCACGUTT
Negative control	Sense: UUCUCCGAACGUGUCACGUTT Antisense: ACGUGACACGUUCGGAGAATT