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Original Paper

Estradiol Modulates the Expression Pattern of Myosin Heavy Chain Subtypes via an ERα-Mediated Pathway in Muscle-Derived Tissues and Satellite Cells

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Key Words
Estrogen receptor • Estrogen receptor selective antagonist • Muscle-derived satellite cells • Myosin heavy chain

Abstract
Background: Muscle-derived satellite cells (MDSCs) express MHC molecules intimately related to muscle function, which is supposed to be affected by local estrogen (E_2) levels. However, cellular targets and molecular mechanisms involved are poorly understood. ***Methods:*** Genioglossus (GG) muscle tissues and MDSCs were derived from SHAM, ovariectomized or ovariectomized and 17 β-estradiol injected rats (n=10 / group). ERα, ERβ, MHC expression and underlying regulatory mechanisms were investigated by RT-PCR, western blot and immunohistochemistry, inter alia upon selective antagonist exposure and Si-RNA transfection. MDSC viability and cell cycle were examined by MTT and flow cytometry. ***Results:*** E_2 upregulated MHC-I and downregulated MHC-IIb expression in MDSCs. E_2 mediated effects on these molecules were inhibited by ERα-selective antagonist MPP and si-ERα, whereas they persisted upon exposure to ERβ-selective antagonist PHTPP. ERα was significantly higher expressed in muscle tissues compared to ERß. ER positive stainings were fewer in the ovariectomized than in the SHAM group. Injection of E_2 only increased the positive staining of ERα, but not of ERβ. ***Conclusion:*** Results suggest that E_2 regulates MHC expression mainly through an ERα-mediated pathway with opposing effects on MHC-I and MHC-IIb. Thus, different hormonal processes that impact muscular pathophysiology presumably govern the functional properties of these molecules.

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Introduction

Skeletal muscle-derived satellite cells (MDSCs) function as a primary source of myogenic cells required for post-natal muscle growth, repair, and regeneration through donating nuclei for the repair of existing fibers or for the formation of new muscle fibers [1, 2]. Recently, E_2 treatment has been shown to stimulate proliferation of cultured bovine satellite cells (BSCs) [3]. Reinecke et al. proved that MDSCs express skeletal muscle-specific myosin heavy chain (MHC) molecules [4], which determine muscular contractile function and fatigue resistance [5]. Slow muscle fiber types qualify for resistance to gravity and fatigue and express MHC-I and different proportions of MHC-IIa. Fast fiber types that can be easily fatigued, maintain body movement and intensive activities mainly express MHC-IIb and MHC-IIx [6-8]. Moran et al. showed that the myosin binding dynamics are altered in ovariectomized mice, leading to a reduction in titanic force that is restorable by E_2 replacement [9]. Furthermore, shifts in the MHC composition towards increased MHC-I expression in the soleus (SoI) and MHC-IIa expression in the extensor digitorum longus (EDL) as much as reduced expression levels of MHC-IIb in the EDL have also been reported following ovariectomy (OVX) in rats [10]. These findings suggest that the expression pattern of MHC subtypes, which determines the function of the skeletal muscle, is related to the E_2 level.

Furthermore, studies identified that hormone replacement therapy (HRT) increases the tonic and inspiratory phasic activity of the genioglossus (GG) muscle in postmenopausal women [11], thus preventing pharyngeal collapse especially during sleep. According to these studies, estrogen (E_2) might impact the contractile function of the GG, but potential cellular targets and molecular mechanisms involved have not been identified yet.

E_2 elicits its biological responses via estrogen receptor (ER) α/β-mediated pathways. The ER is a member of the nuclear receptor (NR) gene superfamily and acts as a ligand-induced transcription factor [12]. As separate genes encode ERα and ERβ, the functions of these two subtypes presumably are slightly different [13, 14]. Hou et al. reported that the GG muscle expresses both ER subtypes and that the contractility of the GG can be accentuated by E_2 [15]. Another group reported an increased muscle fatigue resistance in ovariectomized rats due to an up-regulated expression of ERs by phytoestrogen genistein [16].

It was the aim of our study to investigate the cellular and molecular mechanisms of E_2 dependent effects on MHC subtype expression in MDSCs and to analyze which ER subtypes play the vital role in these regulatory pathways in muscle tissues.

Materials and Methods

Animal models

Thirty 6-week-old female Sprague-Dawley rats (155.4 g ±5.16 g) were maintained under controlled conditions and illumination (12 h light, 12 h dark). The rats were divided into 3 groups comprising 10 animals each: (1) SHAM-operated (SHAM) (2) ovariectomized (OVX) and (3) ovariectomized and 17β-estradiol injection (Sigma-Aldrich, St.Louis, MO, USA). From the second week after being ovariectomized, daily subcutaneous injection was performed with 17β-estradiol (20 μg/kg per day) for 8 weeks. In the SHAM and OVX group, oil was injected instead of 17β-estradiol. The animals in the OVX group were anesthetized with pentobarbitone sodium (40 mg/kg, I.P.) for bilateral removal of the ovaries. In the SHAM group, the abdominal walls were incised, but the ovaries were retained. Animal protocols were approved by the Animal Care Committee of Fourth Military Medical University (License Number: SCXK 2007-007). Each of the following experiments was run in triplicate and comprised n=10.

Immunohistochemical staining

Tissues derived from each of the three experimental groups were embedded in 4% paraformaldehyde, cut in 5 μm sections, autoclaved for 15 min at 100°C and blocked for endogenous peroxidase activity with hydrogen peroxidase. After inhibition of nonspecific reactions with 10% goat serum, the primary antibodies, mouse anti-ERα (dilution 1:100; ab-2746, Abcam, Cambridge, MA USA) and rabbit anti-ERβ (dilution 1:100; ab-3577, Abcam) were incubated overnight at 4°C. Subsequently, the secondary antibody

was incubated for 1 h previous to DAB chromogenic staining according to the manufacturer's instructions. Samples were counterstained with Mayer`s haematoxylin and cover slipped for light microscopical analysis. In order to prove the specificity of the immunoreactions, negative controls were carried out by omitting the primary antibody and using TBS/BSA instead. Images were captured by transmitted-light microscopy. For quantification of the immunostainings, 3 sections from each animal were selected and 5 random fields of each section were captured. The data are expressed as the average area of ER-positive nuclei per group.

Isolation and primary culture of GG MDSCs

Isolation and purification of GG MDSCs were performed using a pre-plating technique as previously described [17]. GG muscle tissues were obtained from the 3 experimental groups and from a control group comprising 10 4-month old rats without any treatment by stripping off the visible connective and fat tissue for dissection into 1 mm^3 fragments with sharp scissors. Enzymatic dissociation was performed by serial digestion of the minced muscles in 0.2% collagenase type IX solution (Sigma, St. Louis, MO) for 1 h, in 0.3% dispase (GIBCO-BRL, Gaithersburg, MD) for 45 min, and in 0.1% trypsin (Life Technologies, Rockville, MD) for 30 min. After enzymatic dissociation, the muscle cells were centrifuged and resuspended in proliferation medium (PM) consisting of DMEM supplemented with 10% fetal bovine serum, 10% horse serum, 0.5% chick embryo extract, and 1% penicillin–streptomycin (GIBCO-BRL). Differentiation medium consisted of DMEM supplemented with 2% horse serum and 1% penicillin–streptomycin. Different populations of MDSCs were isolated based on their adhesion characteristics. MDSCs from passages 2-4 were pooled and grown to 60-80% confluence in PM to prevent initiation of myogenesis.

Immunofluorescence staining

GG MDSCs (passage 3) were seeded into 24-well plates. After 36 h, cells were gently washed with PBS and fixed with 4% paraformaldehyde for 15 min at 4°C. The fixed cells were permeabilized with 0.2% Triton X-100 in PBS for 10 min and blocked in 5% normal goat serum for 60 min. To identify myogenicity of MDSCs, cells were incubated for 2 h with mouse-anti Myod1 (dilution 1:100; Invitrogen, CA, USA) and mouse anti-α-sarcomeric actin (dilution 1:100, Invitrogen) primary antibodies.

Proliferation assays

To examine MDSC proliferation, $5x10^3$ cells/well were cultured in 96-well plates. A 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) assay was carried out for 7 d according to the manufacturer´s protocol (Sigma). Absorbance was determined at 490 nm with a microplate reader (Bio-TEK Instruments, Winooski, VT, USA).

Furthermore, cell cycle analysis was performed on MDSCs. In brief, single cell suspensions were harvested and fixed in ice-cold 75% ethanol 4°C for 24-48 h, washed twice with PBS, stained with 100mg/ml propidium iodide at 4°C for 30 min and subjected to cell cycle analysis using an Elite ESP flow cytometer (Beckman Coulter, Fullerton, CA, USA).

Selection of the effective dose of E2

$5x10^3$ MDSCs/well were cultured in 96-well plates and exposed to different doses of 17β-estradiol (10^{-9}M, 10^{-8}M, 10^{-7}M and 10^{-6}M). In order to investigate the proliferation of MDSCs, a MTT assay was carried out for 7 d according to the manufacturer´s protocol (Sigma). mRNA of MDSCs was collected after 17β-estradiol treatment for 24 h and Real-time PCR analyses were conducted.

Selective ER antagonists

MDSCs were treated with ER selective antagonists to determine the sensitivity of the ER subtypes to E_2. The ERα antagonist MPP dihydrochloride (Tocris Bioscience, UK) and the ERβ antagonist PHTPP (Tocris Bioscience, UK) were added to MDSCs 1 h previous to E_2 treatment at the concentration 100nM. The dose was chosen according to the previously conducted titration experiment for E_2. Cells were harvested for mRNA analysis after a treatment period of 24 h and for protein analysis after a treatment period of 48 h.

Transfection assay

MDSCs at 50% confluence were transfected with the SiPORT NeoFX Transfection Agent (Ambion, AM4510, USA) at a concentration of 50 nM. Cells were harvested 24 h after transfection for mRNA analysis

Table 1. Primers for siRNA transfection. Si: SiRNA

Prime name	Target Sequence Sense (5' to 3')
Si-ERα1	GATAAGAACCGGAGGAAGAGT
Si-ERα2	GTAAATGTGTAGAAGGCAT
Si-ERα3	GCAGCAGCGAGAAGGGAAACA
Si-ERβ1	TGAGCAAAGCCAAGAGAAA
Si-ERβ2	TCACTAAGCTGGCGGACAA
Si-ERβ3	GAGAGACACTGAAGAGGAA
Negative control	UUCUCCGAACGUGUCACGUTT

Table 2. Primers for Real-time PCR

Primer name	Sequence (5' to 3')	Temperature (°C)	Length (bp)
β-actin	F: 5'-GGAGATTACTGCCCTGGCTCCTA-3' R: 5'-GACTCATCGTACTCCTGCTTGCTG-3'	60	150
Pax7	F: 5'-GCTCAGAATCAAGTTCGGGAAG-3' R: 5'-GCTCAGCCGTGAATGTGGTC-3'	60	206
Myod1	F: 5'-CTGATGGCATGATGGATTACAG-3' R: 5'-GGACACTGAGGGGTGGAGTC-3'	60	301
CCND1	F: 5'-AGGAGCAGAAGTGCGAAGAGG-3' R: 5'-GCGGATAGAGTTGTCAGTGTAGATG-3'	60	196
ERα	F:5'-TTATGGGGTCTGGTCCTGTGA -3' R: 5'-TCGGCGGTCTTTTCGTATC -3'	60	193
ERβ	F: 5'-TGCTGGATGGAGGTGCTAATG -3' R: 5'-GAGGTCGGGAGCGAAAATG-3'	60	81
MHC I	F: 5'-GGGCAAACAGGCATTCACTC -3" R: 5'-TTCCCGCAGAAGGTCACAG -3'	60	127
MHC IIa	F: 5'-CAAGGGGCAAGCAAGCAT-3' R: 5'-TTCCCGAAGCAGGTCACAGT-3'	60	131
MHC IIb	F: 5'-TTCCCGAAGCAGGTCACAGT-3' R: 5'-CACAGAAGAGCCCCGAATAAGT-3'	60	132

and 48 h after transfection for protein analysis. siRNAs of ER obtained from Gene-Pharma (ShaiHai, China) were chemically modified (2'-O-Methyl) oligos, whose sequences are listed in Table 1.

Real-time PCR

Total mRNA concentrations of GG muscle and MDSCs samples were determined after extraction with Trizol reagents (Invitrogen, CA, USA). 5 μg of total mRNA was reverse transcribed using reverse transcriptase (Promega, WI, USA) according to the manufacturer's instructions. Real-time PCR was performed with the Taq-Man system (ABI7500 System) and quantification was effectuated by normalizing target gene expression to the housekeeping gene β-actin. Primer sequences, lengths and annealing temperatures are headed in Table 2.

Western blot

MDSCs were harvested in RIPA lysis buffer (Beyotime Co., Shanghai, China) on ice for 1 h and centrifuged for 20 min at 12.000 g. Total and nuclear proteins were extracted with the Nuclear Extraction Kit according to the manufacturer's protocol (Millipore, Billerica, MA). Protein concentrations of the supernatants were determined using the BCA assay. The cell protein extracts were separated on a 8-12% acrylamide gel and transferred to a PVDF membrane. Antibodies were used as follows: ERα (1:500; ab-2746, Abcam, Cambridge, England), ERβ (1:500; ab-3577, Abcam), Myosin light chain (1:300; M4401, Sigma, USA), MYH9 (myosin heavy chain IIa; 1:100; sc-47199, Santa Cruz Biotechnology, Santa Cruz, CA, USA), MYH10 (myosin heavy chain IIb) (1:100; sc-47204, Santa Cruz Biotechnology), histone Deacetylase 1 (1:1000; 2062, Cell signal; USA) and β-actin (1:1000; A5441, Sigma). The signals were detected using the ECL Kit after incubation with the secondary antibody (dilution 1:2000; CoWin Bioscience Co., Beijing, China).

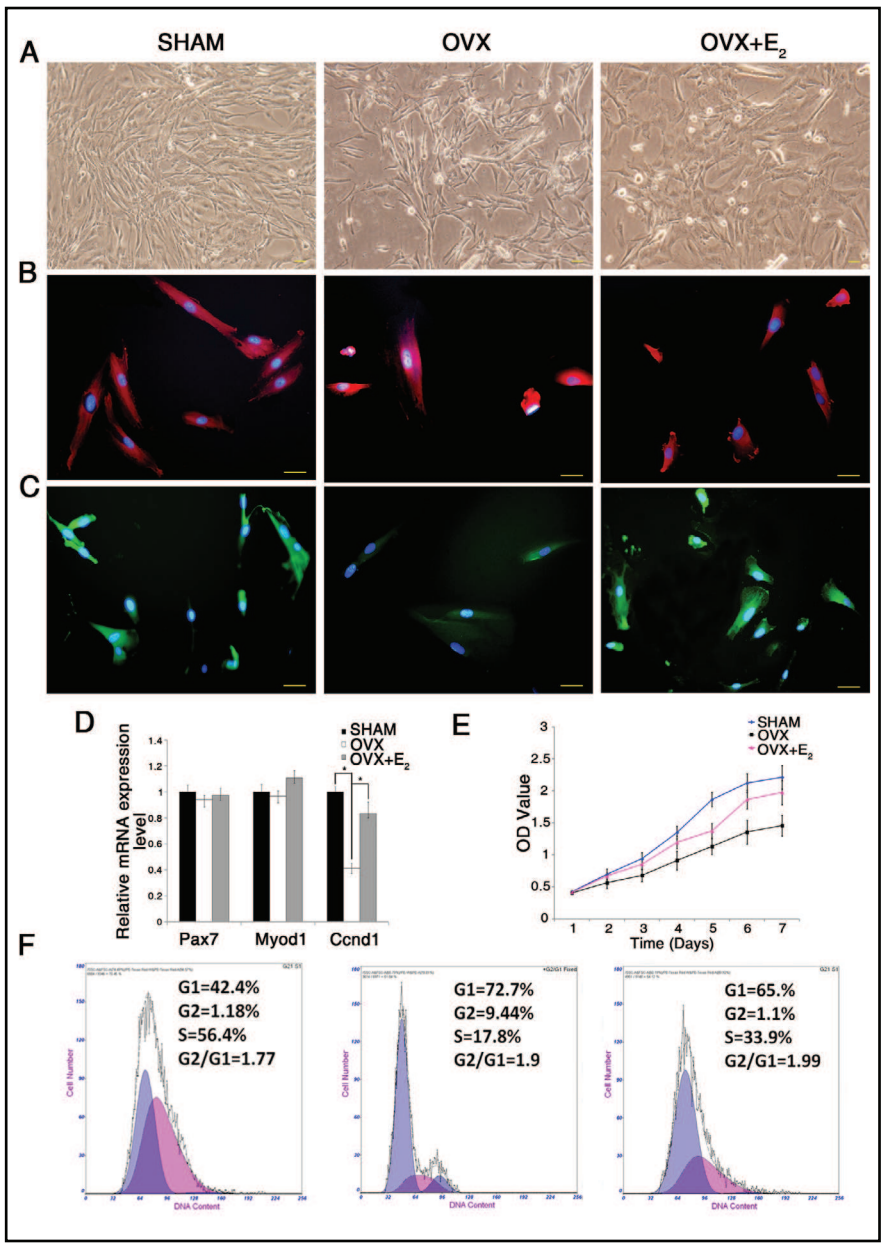

Fig. 1. Identification of rat GG derived MDSCs in the SHAM, OVX, OVX+E_2 group. (A) Microphotograph of rat GG MDSC morphology at 60-80% confluence (200×). Bar is 50 μm. (B) Immunocytochemical staining of cellular actin expression (400×). Bar is 100 μm. (C) Immunocytochemical staining of Myod1 expression (400×). Bar is 100 μm. (D) Gene expression analysis of satellite-related genes Pax7, Myod1 and CCND1 normalized to β-actin. Data are shown as mean ±SD. * P < 0.05. n=10. Each experiment was run in triplicate.(E) MDSC viability investigated by MTT assay. (F) Flow cytometric identification of MDSC proliferation rates by means of cell cycle assay. n=10. Each experiment was run in triplicate.

Statistical analysis

Statistical evaluation was performed using the independent two-tailed Student's t test for detection of statistically significant differences. Values were calculated from n=10 for each experimental group and are expressed as mean ± SD. Experiments were run in triplicate. *P*< 0.05 was considered statistically significant. Analytic tests were conducted with SPSS 17.0 software.

Results

Identification of GG MDSCs

To verify the myogenic origin of GG derived MDSCs, cell cultures were maintained under proliferation and differentiation supporting conditions for 12 d. Noticeably, the cells showed a tendency to directional growth when 60-80% confluence was reached (Fig. 1A). Immunofluorescence staining revealed that cultures at passage 3 were characterized by the

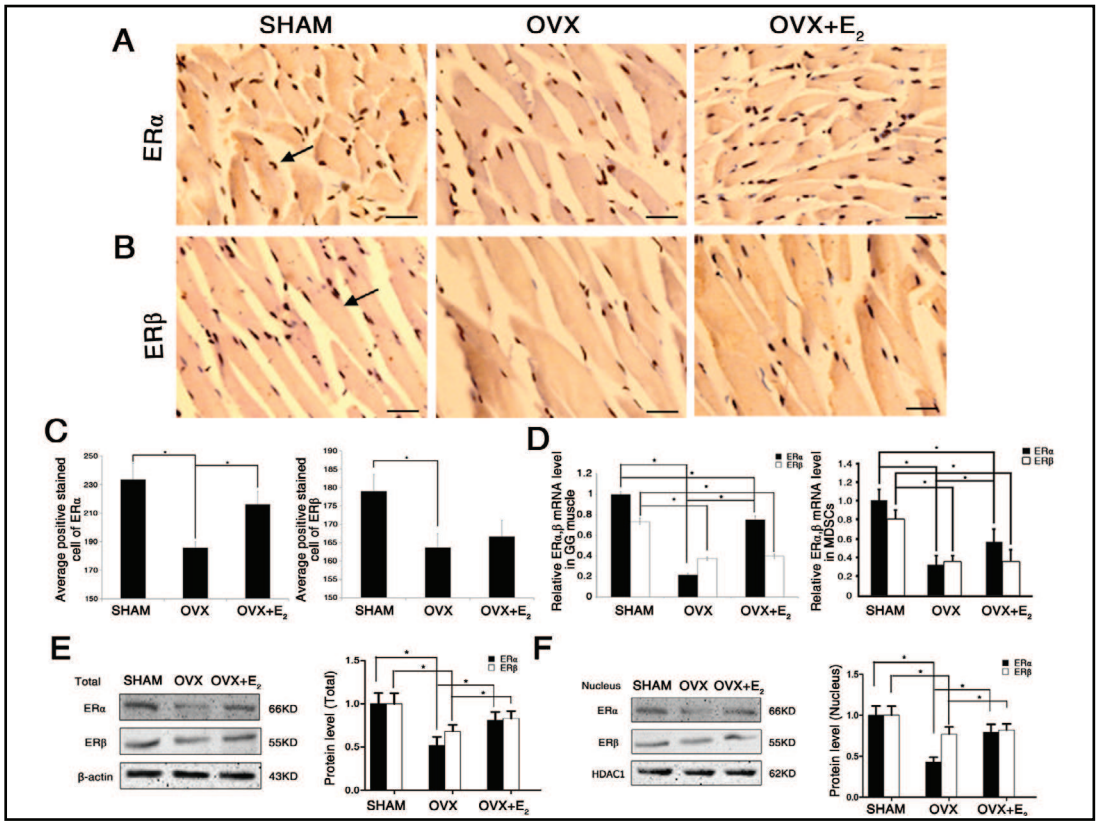

Fig. 2. Expression pattern of ERα and ERβ in GG muscle tissues and in MDSCs of the SHAM, OVX, OVX+E_2 group. (A-B) Immunohistochemical staining for ERα (A) and ERβ (B) in GG muscle tissues (Arrows=ERα / ERβ positive cells). (200×). Bar is 50 μm. (C) Quantification of the immunostainings in GG muscle tissues for ERα and ERβ positive stained cells. 5 random fields were captured of each section. Data are expressed as the average area of ER-positive nuclei per group. (D) Gene expression of ERα and ERβ in GG muscle tissues and in MDSCs analyzed by Real-time PCR. Data are shown as mean ±SD. * $P < 0.05$. n=10. Each experiment was run in triplicate. (E, F) Total and nuclear protein expression levels of ERα and ERβ in MDSCs analyzed by western blot. The relative intensity of the protein expression levels was normalized to β-actin. Data are shown as mean ±SD. * $P < 0.05$. n=10. Each experiment was run in triplicate.

presence of sarcomeric actin and skeletal muscle Myod1. All cultures were 80-90% positive for these markers (Fig. 1B-C).

Real-time PCR showed that Pax7 and Myod1 expression levels did not significantly differ among the 3 groups investigated (Fig. 1D), suggesting that pure myogenic progenitors could be obtained from the primary culture of rat GG muscle cells. MTT assay demonstrated that MDSCs isolated from the OVX group exhibited the lowest viability compared to the other groups, which was obviously increased after injection of E_2 in the OVX+E_2 group, but still remained lower than in the SHAM group (Fig. 1E). Furthermore, cell cycle analyses via flow cytometry proved that the proliferation patterns corresponded the data on cell viability, and the expression pattern of CCND1 as a marker of the cell cycle supported these findings as well (Fig. 1D, F).

ERα cellular and muscle tissue expression is significantly higher than ERβ expression

Immunohistochemical stainings featured ERα and ERβ positive reactions within GG muscle tissues. The average area of ERα positive staining was significantly higher compared to ERβ. Both ERα and ERβ positive stainings were fewer in the OVX group than in the SHAM group. However, injection of E_2 only increased the positive staining of ERα, but not of ERβ in GG muscle tissues (Fig. 2A-C).

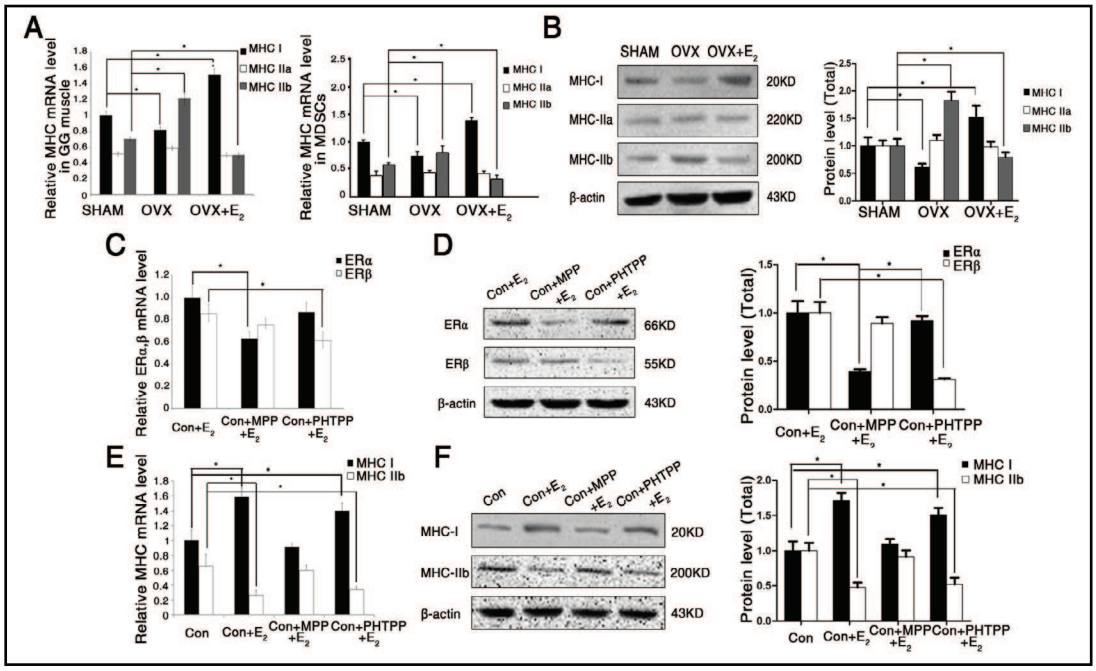

Fig. 3. E_2 regulates the expression pattern of MHC subtypes mainly through an ERα-mediated pathway. (A) MHC subtype gene expression in the SHAM, OVX, OVX+E_2 group in GG muscle tissues and in MDSCs. Data are shown as mean ±SD. * $P < 0.05$. n=10. Each experiment was run in triplicate. (B) Western blot of MHC subtype expression in SHAM, OVX, OVX+E_2 derived MDSCs. The relative intensity of the protein expression levels was normalized to β-actin. Data are shown as mean ±SD. * $P < 0.05$. n=10. Each experiment was run in triplicate. (C, D) ERα and ERβ expression in MDSCs analyzed by Real-time PCR (C) and western blot (D) after application of selective antagonists of ERα and ERβ. Normalization to β-actin. Data are shown as mean ±SD. * $P < 0.05$. n=10. Each experiment was run in triplicate. (E, F) Expression levels of MHC-I and MHC-IIb analyzed by Real-time PCR (E) and western blot (F) after treatment of MDSCs with or without E_2 (1×10^{-7} mol/L) in the presence of the ER-selective antagonists MPP and PHTPP for 3 d. Normalization to β-actin. Data are shown as mean ±SD. * $P < 0.05$. n=10. Each experiment was run in triplicate.

Gene expression patterns of ERα and ERβ in both GG muscle tissues and MDSCs confirmed the results of the immunochemical stainings of the muscle sections. ERα was higher expressed than ERβ in the SHAM group. In the OVX group, both ERα and ERβ expression were decreased, which was most pronounced for ERα. After injecting E_2, ERα expression increased nearly 2-fold, while the expression of ERβ was not significantly changed (Fig. 2D). Western blot analysis showed that MDSCs contain full-length ERα and ERβ. Although the ERs were expressed throughout the cells, the nuclear level of ERα was more affected by E_2 injection than the total protein level. These expression patterns of ERα and ERβ were consistent with the results of the Real-time PCR analyses (Fig. 2E-F). In summary, the expression level of ERα was significantly higher than that of ERβ in both GG muscle tissue and cells. Furthermore, the exogenous estrogen level affected the expression of ERα more pronounced than the one of ERβ especially in the cellular nuclei.

E_2 regulates MHC subtype expression patterns mainly through an ERα-mediated pathway

As the MHC subtypes are assumed to determine contractile function and fatigue resistance of skeletal muscles, we investigated their expression pattern among the 3 groups in GG muscle tissues and in MDSCs. Gene and protein assays showed that the lowest MHC-I expression was detectable in the OVX group. However, it increased more than 2-fold after injection of exogenous E_2, and this expression level was even higher than the one recorded in the SHAM group. On the other hand, the expression pattern of MHC-IIb revealed an

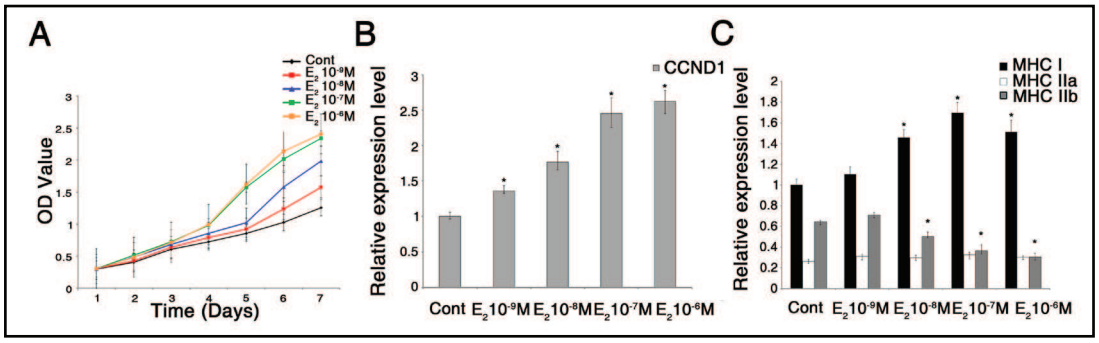

Fig. 4. Effects of E_2 administration on MDSCs are dose-dependent. (A) Cell viability analysis for MDSCs using an MTT assay. (B,C) Gene expression of CCND1, MHCI, MHC IIa and MHC IIb in MDSCs analyzed by Real-time PCR. Normalization to β-actin. Data are shown as mean ±SD. * $P < 0.05$. n=10. Each experiment was run in triplicate.

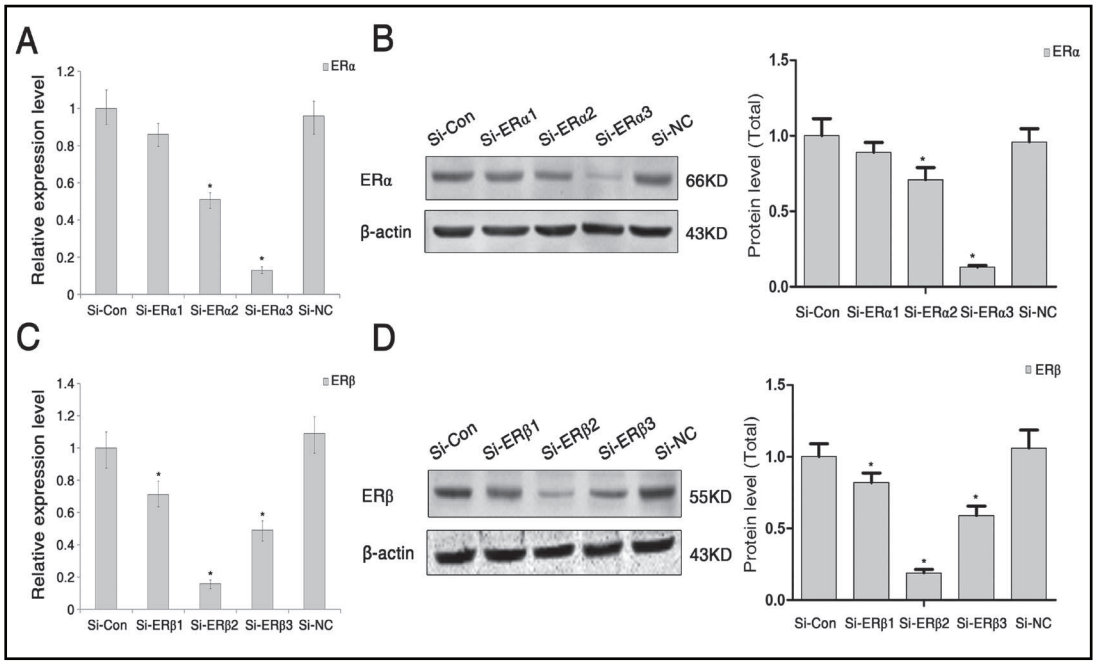

Fig. 5. Transfection efficiency of ERα and ERβ. Gene (A,C) and protein (B,D) expression of ERα and ERß measured by Real-time PCR and western blot. Expression levels were normalized to β-actin. Data are shown as mean ±SD. * $P < 0.05$. n=10. Each experiment was run in triplicate.

opposite tendency. Ovariectomy resulted in a 1.8-fold increase of MHC-IIb expression, while it declined 2.3-fold after injection of E_2 to a level lower than the values seen in the SHAM group. Among the 3 experimental groups, no significant differences in MHC-IIa expression could be denoted (Fig. 3A-B).

To elucidate the cellular mechanism involved in E_2-driven regulation of the transformation of MHC subtypes, we used two ER-selective antagonists, both at a dosage of 100nM, to determine which subtypes are more sensitive to E_2. Among the four E_2 doses applied, 1×10^{-7} M and 1×10^{-6} M E_2 were the most effective doses for promoting MDSCs proliferation (Fig. 4A-B). Furthermore, the expression patterns of MHC under 1×10^{-7} M and 1×10^{-6} M E_2 treatment were close to the levels detected in both tissues and MDSCs directly isolated from the 3 groups (Fig. 4C). Therefore, 1×10^{-7} M E_2 was chosen for further

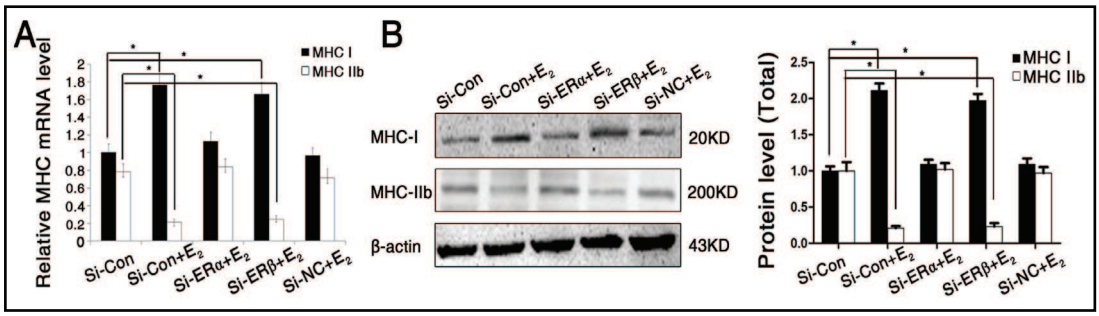

Fig. 6. Downregulation of ERα expression inhibits E_2-induced MHC-I upregulation and blocks E_2 repression of MHC-IIb expression. After downregulation of ERα and ERβ expression via siRNA for 48 h, MDSCs were treated with or without E_2 (1×10^{-7} mol/L) for 3 d and expression levels of MHC-I and MHC-IIb were quantified by Real-time PCR (A) and western blot (B). The expression levels were normalized to β-actin. Data are shown as mean ±SD. * $P < 0.05$. n=10. Each experiment was run in triplicate.

experiments. Analyses showed that each selective antagonist only inhibited its specific ER (Fig. 3C-D). As the E_2 level only affected the expression pattern of MHC-I and MHC-IIb (Fig. 3B), we only assessed the expression of these two subtypes after adding the ERα- and ERβ-selective antagonists to determine whether the effects of E_2 on MHC transformation were receptor-mediated. Absence of an antagonist induced MHC-I upregulation in MDSCs, but by contrast a downregulation of MHC-IIb. Additive of the ERα-selective antagonist MPP blocked these effects on MHC-I and MHC-IIb expression mediated by exogenous E_2. However, the E_2-induced effects on MHC-I and MHC-IIb expression were maintained to some extent after treatment with the ERβ-selective antagonist PHTPP (Fig. 3E-F).

Si-ERα decreased the expression level of ERα 7.7-fold and of ERβ 5.3-fold compared to the control (Fig. 5A-D). Real-time PCR and western blot showed that the E_2-induced effects on the expression of MHC-I and MHC-IIb were inhibited after decreasing ERα expression while they were still maintained after ERβ downregulation (Fig. 6A-B) compared to the E_2-induced control group.

Discussion

Our investigations revealed that E_2 modulates both MHC-I and MHC-IIb expression patterns in MDSCs, presumably via an ERα-mediated pathway. Consequently, regulation of MHC subtypes seems to be dependent on the hormone levels of E_2, which also impacts cellular performance of MDSCs. Estrogen is known to promote proliferation and differentiation of MDSCs that express functional ERs [18] and plays an important role in regulating muscular function [19]. Raising evidence is given that sex-related differences in MDSC performance are a factor for the heterogenity in cell-induced skeletal muscle regeneration, as investigations revealed that female MDCSs display an advanced marker expression profile, whereas male MDSCs show a higher differentation potential upon subject to oxidative stress [20]. Studies revealed that ovariectomy followed by E_2 replacement influences MHC expression patterns that determine muscular contractile speed [16]. MHC-I is predominantly expressed in slow, oxidative skeletal muscle fibers that are important for endurance activities and for resistance against gravity and fatigue. Although MHC I is a standard marker to characterize the differentiation status of satellite cells [21], undifferentiated MDSCs also express this molecule due to their multilineage differentiation potential [17]. This phenomenon is analogous to osteoblast marker genes such as Runx2 and ALP that also expressed when mesenchymal stem cells are cultured in common media, and whose expression levels are significantly increased after the osteogenic induction [22, 23]. Generally, MHC-IIb has the greatest effect on the speed of muscle contraction, while MHC-I has a greater effect on muscular endurance and resistance to fatigue [24].

Besides the fact that gender-specific differences and hormonal levels impact cellular mechanisms, the ER subtypes and their different subtype-selective ligands condition distinct physiological functions on cellular and molecular level as well [25, 26]. In contrast to our results demonstrating that the exogenous E_2 level affects nuclear ERα expression more than ERβ, Velders et al. proved that estrogens affect the MHC composition through a mechanism mainly mediated by ERβ in Soleus and Gastrocnemius [16]. Consequently, different expression patterns of ERα and ERβ as much as diverse biological functions that may entail varying sensitivity to E_2 are likely to exist in diverse muscle tissues. At this, the molecular actions of ERs are certainly overlapping to some extent among the specific tissues, but are in all likelihood also characterized by cell-type intrinsic processes in large part [27]. Moreover, both the transcriptional and posttranscriptional regulation of ER expression and action are guided by complex factors which presumably are determined by the structural and cellular context of the tissue involved. Although both ERs are expressed in skeletal muscles of murines and humans [28-30], the results of the experiments conducted in murines most likely do not exactly reflect human pathophysiology. As a consequence, our results might provide valuable evidence for improving the therapeutic options in some ER-dependent diseases, but their specific role in human muscle tissue needs to be further elucidated.

The findings of our investigations indicate a coherency between MHC subtype expression and estrogen levels mediated by an ERα dependent pathway in muscle-derived tissues and satellite cells. Thus, this complex regulatory system presumably governs various cellular and molecular pathophysiological mechanisms, which remain to be elucidated.

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Conflict of Interest

All authors declare that there is no conflict of interest.

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Errata

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In the article by Guo et al., entitled "Estradiol Modulates the Expression Pattern of Myosin Heavy Chain Subtypes via an ERα-Mediated Pathway in Muscle-Derived Tissues and Satellite Cells" [Cell Physiol Biochem 2014;33:681-691 (DOI: 10.1159/000358644)], is a printing error in the affiliations. The corrected authors and their affiliations are stated correctly here.

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