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Molecules and Cells

Leukotriene B_4 Regulates Proliferation and Differentiation of Cultured Rat Myoblasts via the BLT1 Pathway

Ru Sun[1,2,3], Xueqing Ba[1,3], Lingling Cui[1], Yan Xue[1], and Xianlu Zeng[1,*]

Skeletal muscle regeneration is a highly orchestrated process initiated by activation of adult muscle satellite cells. Upon muscle injury, the inflammatory process is always accompanied by muscle regeneration. Leukotriene B_4 is one of the essential inflammatory mediators. We isolated and cultured primary satellite cells. RT-PCR showed that myoblasts expressed mRNA for LTB_4 receptors BLT1 and BLT2, and LTB_4 promoted myoblast proliferation and fusion. Quantitative real-time PCR and immunoblotting showed that LTB_4 treatment expedited the expression process of differentiation markers MyoD and M-cadherin. U-75302, a specific BLT1 inhibitor, but not LY2552833, a specific BLT2 inhibitor, blocked proliferation and differentiation of myoblasts induced by LTB_4, which implies the involvement of the BLT1 pathway. Overall, the data suggest that LTB_4 contributes to muscle regeneration by accelerating proliferation and differentiation of satellite cells.

INTRODUCTION

Skeletal muscle injury, including trauma, neurological dysfunction or innate genetic defects, if left un-repaired, may lead to loss of muscle mass, locomotive deficiency and in severe cases, death. Maintenance of functional skeletal muscle is conferred by its remarkable ability to regenerate (Chargé and Rudnicki, 2004; Collins, 2006). Adult skeletal muscle regeneration results from activation, proliferation and fusion of myogenic precursor cells, so-called satellite cells (Hawke, 2001).

Upon muscle injury, an inflammatory process accompanies the process of muscle regeneration (Tidball, 2005). It is widely acknowledged that inflammation is functionally beneficial to muscle regeneration (Tidball, 2005). Leukocytes are always involved in phagocytosis of debris and removal of damaged muscle (Grounds, 1987). Moreover, cytokines secreted by leukocytes and damaged tissue can promote the function of muscle satellite cells. Macrophages are attracted by satellite cells and secreted chemotactic factors to stimulate their proliferation and rescue them from apoptosis, which enhances muscle growth (Chazaud et al., 2003). However, the role of inflammatory mediators that are metabolized from damaged plasma membrane, such as prostaglandins and leukotrienes, in the functional regulation of muscle satellite cells has not yet been explored.

In damaged muscle tissue, activation of cytosolic phospholipase A_2 (PLA_2) leads to the liberation of arachidonic acid (AA) from membrane phospholipids. AA can be metabolized into two major classes of eicosanoids, prostaglandins and leukotrienes (Kim and Luster, 2007; Yokomizo et al., 2001). Leukotriene B_4 (LTB_4) has been demonstrated to induce numerous inflammatory functions in leukocytes, including chemotaxis, degranulation and adhesion to endothelial surfaces (Kim and Luster, 2007; Serhan et al., 1996).

According to previous studies, there are two types of LTB_4 receptors, BLT1 and BLT2, with higher and lower binding affinity to LTB_4 on neutrophils, respectively (Kim and Luster, 2007). Even though most previous studies have shown that BLT1 expression is largely limited to leukocytes (Kim and Luster, 2007), it has recently been demonstrated that BLT1 activation in vascular smooth muscle (Bäck et al., 2005), endothelial (Qiu et al., 2006) and neural stem (Wada et al., 2006) cells results in cell proliferation, differentiation, migration and chemokine production, which enhances the inflammatory response or induces neurite outgrowth. The distribution of BLT2 is different in human and mice in spite of their significant shared homology. Human BLT2 is ubiquitously expressed in various tissues, whereas, mouse BLT2 has a more restricted distribution in the small intestine, colon and keratinocytes (Kim and Luster, 2007).
The questions arise as to whether skeletal muscle satellite cells express LTB_4 receptors, and whether the inflammatory mediator LTB_4 contributes to skeletal muscle regeneration by activation of satellite cells. In the present study, we isolated primary muscle satellite cells, and demonstrated expression of BLT1 and BLT2 mRNA in cultured myoblasts, as well as the effects of LTB_4 on proliferation and differentiation of myoblasts.

MATERIALS AND METHODS

Reagents and antibodies

Collagenase type II and dispase were respectively purchased

[1]Institute of Genetics and Cytology, Northeast Normal University, Changchun 130024, China, [2]School of Physical Education, Jilin University, Changchun 130012, China, [3]These authors contributed equally to this work.
*Correspondence: zengx779@nenu.edu.cn

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from Worthington Biochemical Corporation (USA) and BD Biosciences (USA). Ham's F-10 medium and DMEM, fetal bovine serum and horse serum were from Gibco Invitrogen (USA). Leukotriene B_4 (LTB_4) was purchased from Alexis Biochemicals (USA). U-75302 and LY255283, BLT1 and BLT2 inhibitors, respectively, were from Cayman Chemical (USA). RNA extraction agent Trizol was from Invitrogen Corporation. Reverse transcriptase kit (A3802) was from Promega (USA).

Monoclonal antibody against MyoD 5.8A (sc-32758) and goat polyclonal antibody against M-cadherin N-19 (sc-6470) were from Santa Cruz Biotechnology (USA). Monoclonal antibody against β-actin was from Sigma (USA). Horseradish peroxidase (HRP)-conjugated secondary antibodies were from Jackson Laboratory (USA). Calcein AM used for staining and counting of living cells was from Molecular Probe Invitrogen (USA).

Isolation and culture of muscle satellite cells

Isolation and culture of muscle satellite cells were carried out according to previously described protocols, with minor modification. Briefly, 6-8-week-old rats were sacrificed and hind-limb muscles were isolated and collected in PBS with penicillin and streptomycin. The muscle was minced into fine pieces and transferred to digestion buffer (0.5% collagenase typeII, 1% dispase in PBS), incubated for 40 min at 37°C, and mixed during the incubation. Digestion was terminated by adding 10% FBS. Samples were centrifuged at 500 × *g* for 1 min and the supernatant was filtered through a cell strainer, and the suspension was re-centrifuged at 900 × *g* for 10 min. The pellets, containing skeletal muscle satellite cells, were resuspended in complete medium (Ham's F-10 with 20% FBS). After five rounds of pre-plating, cells were plated in 35-mm^2 plates at a density of 1-2 × 10^4 cells/cm^2. After removing the non-adhered cells and debris 24 h later, the cells were cultured in the same medium as used for proliferation for 3 d, and were passaged under differentiation conditions (DMEM with 10% FBS and 10% horse serum) to 14 day.

Immunofluorescence labeling of myoblasts

Cultured satellite cells were washed with PBS, and fixed with 4% formaldehyde/PBS at room temperature for 15 min. After washing three times with PBS, cells labeled for M-Cadherin were incubated with primary antibody at 1:50 dilution for 1 h, whereas, cells labeled for MyoD under the same conditions needed permeabilization in 0.25% Triton X-100/PBS (containing 5 mM EDTA and 2% FBS) for 5 min and washing for three times. After eliminating the excessive primary antibodies with PBS, cells were incubated with FITC-conjugated secondary antibodies at 1:2000 dilution for a further 1 h. After washing three times in PBS, specimens were labeled with DAPI for 5 min. After final washing, fluorescence-labeled cells were observed under the fluorescence microscope (Nikon TE 2000-U, Japan).

Myoblast staining and counting

To investigate the role of LTB_4 on the proliferation of satellite cells, following the removal of non-adhered cells and debris, LTB_4 was added to the growth medium at different concentrations (30, 60, 100 and 150 nM) for 2 d. Normally cultured and LTB_4-treated cells were stained *in situ* with Calcein AM at 1 μg/ml for 20 min, and quantified by Gemini EM (Molecular Devices, USA.) with excitation at 485 nm and emission at 535 nm. Mean fluorescence of five wells in each 48-well plate was determined as the sample's relative cell number ($n = 5$). The value of each sample was expressed as the relative fluorescence index (RFI). Significant differences from the normal controls were determined by one-way ANOVA ($^*P < 0.01$). To determine the receptor that mediated LTB_4 signaling, inhibitors were added at different concentrations 30 min before treatment with 60 nM LTB_4. Cell staining and counting was the same as above.

Measurement of fusion percentage of myoblast

The differentiation of cultured satellite cells was detected as described elsewhere (Yoshida et al., 1996). Briefly, the cells treated with or without LTB_4 were washed with PBS and stained with the Wright-Giemsa method. The fusion index was determined by dividing the total number of nuclei in myotubes by the total number of nuclei counted. At least 100 myotubes and 500 nuclei per condition were analyzed for each assay.

RNA isolation, RT-PCR and real-time PCR

Total RNA of normally cultured and LTB_4-treated myoblasts was extracted by Trizol regent and 2 μg of each sample's RNA was reverse transcribed into cDNA. All samples were subjected to PCR amplification and expression of mRNA was normalized with that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). RT-PCR conditions were: 94°C for 30 s; 58°C for 30 s; 72°C for 30 s; repeated 30 cycles for GAPDH; 25 cycles for β-actin and 35 cycles for MyoD and M-cadherin. Oligonucleotide primers specific for different genes were listed in Table 1.

To detect the effect of LTB_4 on differentiation, LTB_4 was added to the differentiation medium 12 h before days 5 and 8. Quantitative real-time PCR was performed to examine the transcription of MyoD and M-cadherin using the same primers as above for GAPDH, MyoD and M-cadherin. Real-time PCR using SYBR green fluorescence was performed. Each real-time PCR reaction consisted of 1 μl RT product, 12.5 μl SYBR Green PCR Master Mix (PE Applied Biosystems, CA), and 0.5 μM primers. In the ABI Prism 7000 sequence detection system, PCR cycling conditions were as follows: 50°C for 2 min, 95°C for 10 min, 40 cycles at 94°C for 15 s, and 60°C for 1 min. Expression of MyoD and M-cadherin genes was normalized to that of GAPDH. Significant differences were determined by one-way ANOVA ($^*P < 0.01$).

Immunoblotting

To detect protein expression of MyoD and M-cadherin, LTB_4 was added in the medium 24 h before day 5 or day 8. The normally cultured and LTB_4-treated cells were trypsinized and washed with PBS. The pellets were resuspended in lysis buffer containing 1 mM EDTA, 10% glycerol, 0.5% NP-40, 5 mM NaF, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride in PBS at 4°C. The lysates were subjected to 10% SDS-PAGE. The separated proteins were transferred to Immobilon-P membrane and incubated with primary antibodies. HRP-conjugated secondary antibodies against mouse and goat IgG were used to detect the expression of β-actin, MyoD and M-cadherin by employing enhanced chemoluminescence (ECL) (Amersham Bioscience, USA) according to the manufacture's instructions.

RESULTS

Cultured myoblasts express BLT1 and BLT2 mRNA

Cells were activated and began to differentiate upon entering *in vitro* culture, and these differentiated myogenic precursor cells are called myoblasts. In the first 3 days, cells cultured in growth medium maintained in the proliferation stage had no obvious morphological alteration (Fig. 1A). After 6-8 days culture, cells differentiated into myotubes and fused to each other (Fig. 1B). For 12-14 d, cells formed confluent monolayers (Fig. 1C). Immunofluorescence labeling by antibodies against transcrip-

Table 1. Primers used for RT-PCR and real-time PCR

Primer name	Sequence 5′-3′	Product length
GAPDH	Forward: 5′-CCATGGAGAAGGCTGGGG-3′ Reverse: 5′-CAAAGTTGTCATGGATGACC-3′	195 bp
β-Atin	Forward: 5′-GAGGGA AATCGTGCGTGAC-3′ Reverse: 5′-GGAGCCAGGGCAGTAATC-3′	351 bp
BLT1	Forward: 5′-CTG CTGGTGCTGAACTTGG-3′ Reverse: 5′-CCGTGATGGCTTCAAAGAG-3′	401 bp
BLT2	Forward: 5′-ATGAGACGCTGCTGAGTTGGA-3′ Reverse: 5′-AAAGGCCGAGTGACCGCTA-3′	352 bp
MyoD	Forward 5′-GTGCAAGCGCAAGACCACTAA-3′ Reverse 5′-TGCAGACCTTCAATGTAGCGG-3′	182 bp
M-Cdherin	Forward: 5′-GTTCACCAAGGATGAGTTCTTTATGG-3′ Reverse: 5′-AGGATGGTGAACCTGGCCACCCAGTT-3′	125 bp

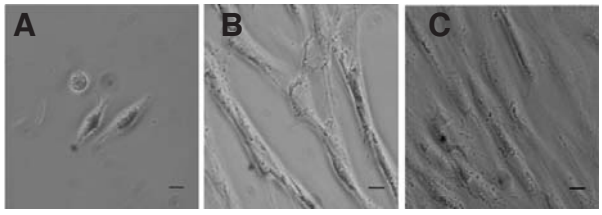

Fig. 1. Differentiation of rat satellite cells *in vitro*. (A) In the first 3 days, cells maintained in the proliferation stage had no obvious morphological alteration. (B) After 6-8 days culture, cells differentiated into myotubes and fused to each other. (C) After 12-14 days culture, cells formed confluent monolayers. Bar, 10 μm.

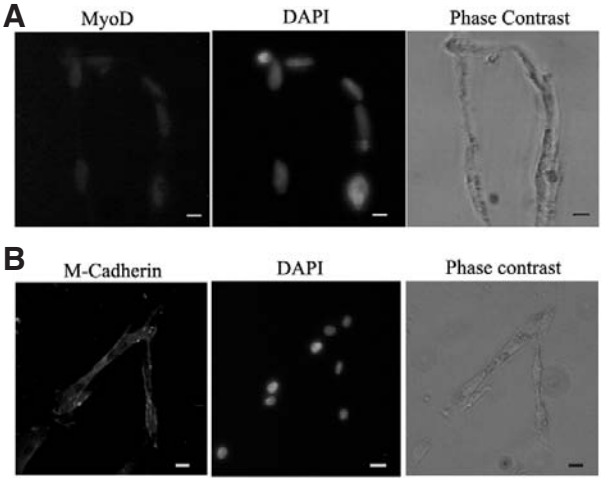

Fig. 2. Immunofluorescence labeling of myoblasts cultured for 6-8 d. (A) MyoD was immunofluorescence-labeled, and nuclei were stained with DAPI. Bar, 5 μm. (B) M-cadherin was immunofluorescence-labeled, and nuclei were stained with DAPI. Bar, 10 μm.

tional factor MyoD and adhesion molecule M-cadherin (marker molecules of differentiated myoblasts) of cells at 6-8 days showed that > 95% of cultured cells were positive and could be used for the following experiments (Figs. 2A and 2B).

None of the previous studies have demonstrated the presence of LTB_4 receptors on muscle satellite cells. To determine whether cultured myoblasts expressed BLT1 and BLT2 mRNA, we chose rat peripheral blood leukocytes as a positive control, since expression of LTB_4 receptors in leukocytes was well known. We first detected expression of BLT1 and BLT2 mRNA in myoblasts cultured for 12 d. While high expression of BLT1 and BLT2 mRNA was observed in rat peripheral blood leukocytes, much lower expression was also detected in these myoblasts (Fig. 3A). With the suspicion that the weak mRNA expression was due to long-term culture, we performed dynamic analysis of expression of BLT1 and BLT2 mRNA at different time intervals. BLT1 and BLT2 mRNA expression increased and reached a maximum on day 8, and then dramatically decreased on day 12 when the cells formed confluent monolayers (Fig. 3B). Moreover, the expression level of BLT2 during the whole culture period was lower than that of BLT1.

LTB_4 expedites proliferation of cultured myoblasts

Does LTB_4 have an effect on proliferation of cultured myoblasts? Different concentrations of LTB_4 (30, 60, 100 and 150 nM) were added to the growth medium at the beginning of culture and the cells were treated for 2 d. The cells were stained with Calcein AM at 1 μg/ml for 20 min, and quantified by a CytoFluor microplate reader with excitation at 485 nm and emission at 535 nm. The relative number of each sample was represented by the ratio of its fluorescence intensity to that of normally cultured cells. The results showed that the number of cells in all LTB_4-treated groups was significantly more than that in the normally cultured group, and proliferation reached a maximum of approximately a six-fold increase with 60 nM LTB4 (Fig. 4A). This indicated that LTB_4 expedited myoblast proliferation.

LTB_4 expedites myoblast differentiation

Does LTB_4 influence differentiation of cultured myoblasts in addition to promoting their proliferation? We first detected the effect of LTB_4 on the fusion of cultured satellite cells. The fusion index of myoblast treated with LTB_4 for 1, 3, or 5 d obviously increased compared with that of untreated cells (Figs. 4B and 4C), and the morphology of those treated cells was also largely changed (supplemental data). Moreover, we selected MyoD and M-cadherin as targets because they are specific myogenic molecules during myoblast differentiation (Arnold and Winter, 1998; Wróbel et al., 2007) and analyzed the dynamic expression of MyoD and M-cadherin during *in vitro* culture. The result

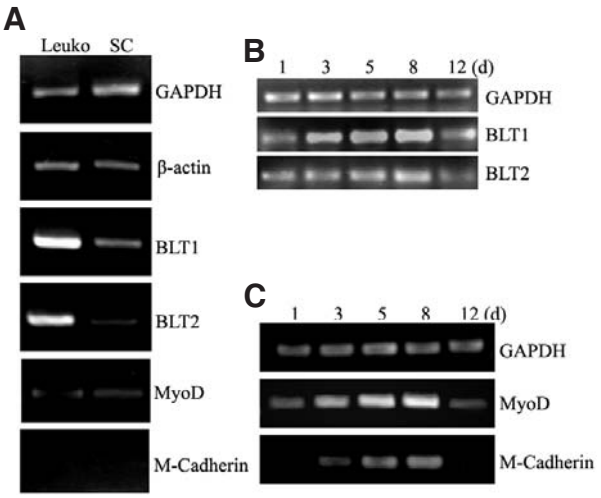

Fig. 3. Expression of mRNA in cultured myoblasts. (A) mRNA expression in rat peripheral blood leukocytes and myoblasts cultured for 12 d. (B) mRNA expression of BLT1 and BLT2 in myoblasts cultured for the indicated time. (C) mRNA expression of MyoD and M-cadherin in myoblasts cultured for the indicated time.

showed that satellite cells isolated and cultured for 1 d had weak mRNA expression for MyoD and undetectable expression for M-cadherin. On day 8, the mRNA levels of the two genes reached a maximum, and on day 12, it dramatically decreased to the level at the beginning of culture (Fig. 3C). When the cells were cultured with LTB_4, the mRNA expression of two molecules reached the maximum on day 5, implying the accelerating of the differentiation (supplemental data). Therefore, we chose days 5 and 8 as two checkpoints to detect if the expression of MyoD and M-cadherin was affected by LTB_4. The result showed LTB_4 clearly increased mRNA expression for MyoD and M-cadherin in myoblasts on day 5, and markedly decreased expression on day 8 (Figs. 5A and 5C). The effect of LTB_4 on protein expression in myoblasts on day 5 and day 8 were also detected, and the similar protein expression patterns of MyoD and M-cadherin with that of mRNA were observed after LTB_4 treatment for 24 h (Figs. 5B and 5D). The results indicated that LTB_4 promoted the differentiation process revealed by the expression alteration of MyoD and M-cadherin.

BLT1 mediates the effect of LTB_4 on myoblasts

To determine which receptor mediated LTB_4 signaling, we studied the effect of U-75302 and LY255283 (respective inhibitors of BLT1 and BLT2) on myoblast proliferation and differentiation. The inhibitors were added at different concentration 30 min before treatment with 60 nM LTB_4 as describe in *Materials and Method* and cell staining and counting was the same as above. U-75302 rather than LY255283 significantly eliminated LTB_4-induced myoblast proliferation and its minimal effective dose was almost 1 μM (Figs. 4D and 4E). Furthermore, the two inhibitors were added 30 min before LTB_4 treatment, and after 12 h co-incubation, mRNA of MyoD and M-cadherin in myoblasts on day 8 were detected by RT-PCR and real-time PCR. U-75302 but not LY255283 obviously rescued mRNA expression for MyoD and M-cadherin (Fig. 5E). Overall, the data suggest that LTB_4 promotes the proliferation and differentiation of myoblasts via a BLT1-mediated pathway.

DISCUSSION

Skeletal muscle regeneration is a highly orchestrated process initiated by the activation of adult muscle satellite cells, which are normally quiescent, but in response to injury, they proliferate, differentiate and fuse to repair or replace damaged myofibers (Chargé and Rudnicki, 2004; Collins, 2006). It is becoming established that, along with this regeneration process, inflam-

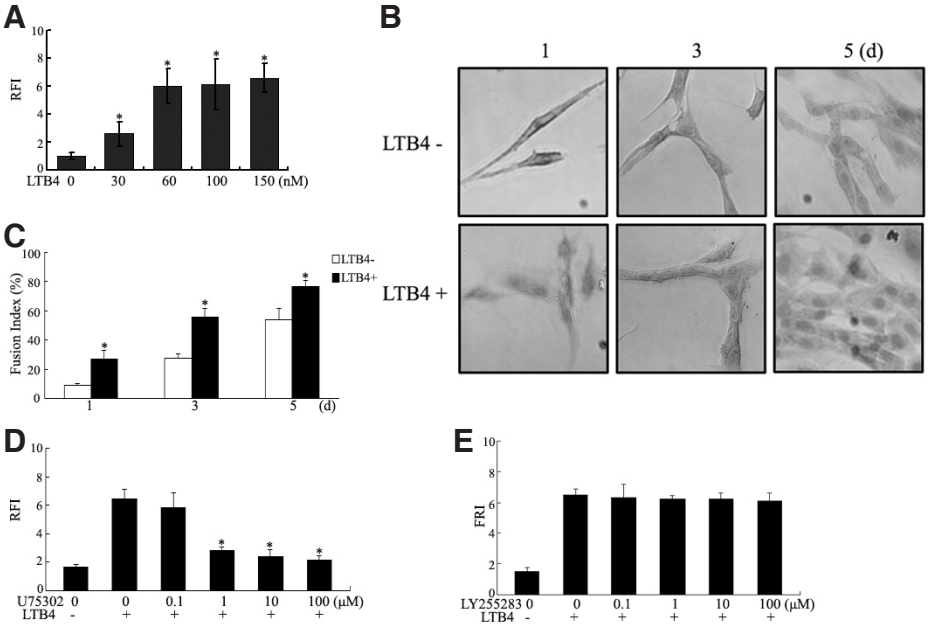

Fig. 4. LTB_4 expedites myoblast proliferation and differentiation. (A) Effects of LTB_4 on myoblast proliferation. Normally cultured and LTB_4-treated cells were stained with Calcein AM, and quantified by a CytoFluor microplate reader with excitation at 485 nm and emission at 535 nm. The mean fluorescence intensity from five wells of cells in 48-well plates was determined as the sample's relative cell number. The value of each sample was expressed as relative fluorescence index (RFI). (B) The myoblasts were cultured in the presence or absence of LTB_4 for the indicated time, and the fusion status of myoblast was showed by Wright-Giemsa staining. (C) The effect of LTB_4 on the fusion percentage of myoblast. (D, E) Effects of co-incubation of LTB_4 and its inhibitors on proliferation of primary satellite cells. Inhibitors were respectively added 30 min before treatment with 60 nM LTB_4. The dose of inhibitor was used as indicated. Cell staining and counting was performed as above. The significant difference of values was determined by one-way ANOVA (*$P < 0.01$), and the data represent the mean ± SD from three independent experiments.

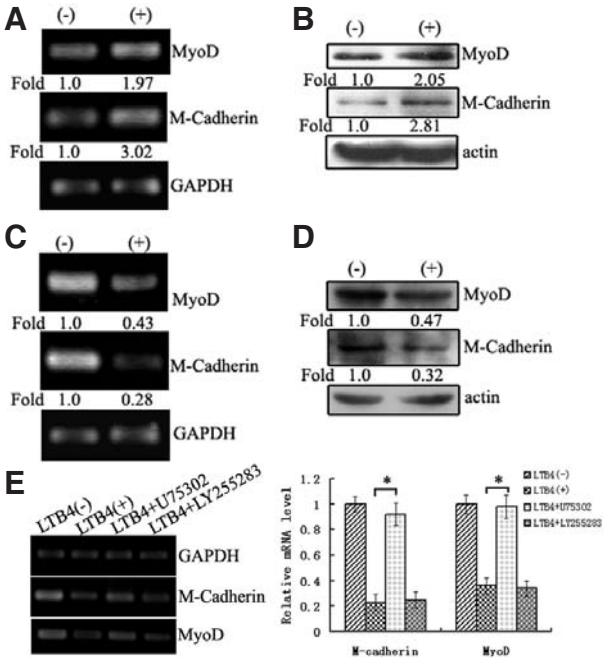

Fig. 5. The effects of LTB_4 on the expression of myogenic markers of myoblast. (A) Effects of LTB_4 on mRNA abundance of MyoD and M-cadherin in myoblasts cultured for 5 d. LTB_4 was added to differentiation medium 12 h before day 5 at a concentration of 60 nM. (B) Effects of LTB_4 on protein expression of MyoD and M-cadherin in myoblasts cultured for 5 d. LTB_4 was added to differentiation medium 24 h before day 5 at a concentration of 60 nM. Expression of MyoD and M-cadherin was detected by ECL. Immunoblotting of β-actin, MyoD and M-cadherin were located at 42, 45 and 97 kDa, respectively. (C) Effects of LTB_4 on abundance of MyoD and M-cadherin mRNA in myoblasts cultured for 8 d. The experiments were performed as (A). (D) Effects of LTB_4 on protein expression of MyoD and M-cadherin in myoblasts cultured for 8 d. The experiments were performed as (B). The blot analysis of (A)-(D) was quantified by densitometry and data were normalized with respect to controls. Data are representative of three independent experiments. (E) U75302, but not LY255283, restored expression of MyoD and M-cadherin mRNA in myoblasts on day 8. Inhibitors were added at a concentration of 10 μM 12 h before day 8, 30 min ahead of LTB_4 treatment. Normally cultured, LTB_4-treated myoblasts and those co-incubated with inhibitors were collected. RT-PCR and real-time PCR were performed. Left panel, electrophoreses of RT-PCR product; right panel, quantitative assay by real-time PCR. The significant difference of values was determined by one-way ANOVA ($^*P < 0.01$), and the data represent the mean ± SD from three independent experiments.

matory elements promote the myogenic activity of satellite cells (Tidball, 2005).

LTB_4 is an essential inflammatory mediator, and the role of LTB_4 in muscle regeneration has not been investigated. None of the previous studies have confirmed the expression of BLT1 and BLT2 in satellite cells or myoblasts, therefore, we detected their mRNA expression in primary myoblasts, and RT-PCR showed that BLT1 and BLT2 mRNA was expressed in these cells (Figs. 3A and 3B).

Despite neither of these two proteins being detected, addition of LTB_4 to the medium promoted the proliferation of cultured satellite cells, which implies the existence of receptors to mediate LTB_4 function (Fig. 4A). The fact that U-75302, but not LY255283, reduced the acceleration of LTB_4-induced myoblast proliferation (Figs. 4D and 4E), suggests the involvement of the BLT1 pathway.

To investigate the impact of LTB_4 on myoblast differentiation, we investigate the effect of LTB_4 on the fusion of myoblast, fusion index of myoblast treated with LTB_4 obviously increased (Figs. 4B and 4C). We additionally focused on two molecules closely related to skeletal muscle development. MyoD is a transcriptional factor in myogenic regulation and M-cadherin is one of its targets. The family of myogenic regulatory factors (MRFs), including Myf5, MyoD, myogenin and MRF4, are critical for the myogenic lineage progression of satellite cells and their myoblast progeny (Collins, 2006). It is generally accepted that Myf5 and MyoD act early to establish myoblasts, and myogenin mediates their terminal differentiation. MRF4 is also assumed to exert late functions in the myogenic pathway (Arnold and Winter, 1998). MyoD activation may lead to robust expression of several muscle-specific genes such as myogenin, M-cadherin, and myosin heavy and light chains (Berkes and Tapscott, 2005). M-cadherin is a calcium-dependent adhesion molecule in the cadherin family, which plays an important role in myoblast recognition, adhesion and fusion. In normal adult muscle, the protein is localized at the satellite cell-myofiber interface (Bornemann and Schmalbruch, 1994). M-cadherin mRNA was below the detection level in an *in situ* hybridization study of satellite cells from normal adult mouse muscle, but it was detected in regenerating muscle (Moore and Walsh, 1993). Moreover, its expression is restricted to myoblasts, and is downregulated after fusion of myoblasts into myotubes, and is barely detectable in fused myotubes (Irintchev et al., 1994).

The dynamic analysis of expression of MyoD and M-cadherin in cultured myoblasts showed that satellite cells isolated and cultured for 1 d had weak expression of MyoD and undetectable expression of M-cadherin. On day 8, expression of the two genes reached a maximum, and on day 12, their expression dramatically decreased to the level at the beginning of culture (Fig. 3C). These *in vitro* dynamic alterations of mRNA of MyoD and M-cadherin were similar to those seen *in vivo* (Arnold and Winter, 1998; Moore and Walsh, 1993; Irintchev et al., 1994).

The effect of LTB_4 on expression of MyoD and M-cadherin was analyzed. Treatment with LTB_4 significantly increased MyoD and M-cadherin mRNA in myoblasts on day 5, and dramatically decreased them in myoblasts on day 8 (Figs. 5A and 5C), when cells began to fuse into myotubes. The protein level of these two molecules in myoblasts on day 5 and day 8 showed the similar patterns with those of mRNA after 24 h of LTB_4 treatment (Figs. 5B and 5D). The effects of LTB_4 on expression of MyoD and M-cadherin indicated acceleration of myoblast differentiation, which suggests that LTB_4 promotes myoblast differentiation in muscle regeneration. U-75302, but not LY255283, restored the mRNA level of MyoD and M-cadherin in myoblasts on day 8 (Fig. 5E), which suggests that BLT1 mediates LTB_4 function.

It was surprising that a low level of MyoD mRNA was detected, in peripheral blood leukocytes, while the absence of M-cadherin was as expected (Fig. 3A). Although many studies have established that the majority of postnatal satellite cells are from the somites (Gros et al., 2005), it has been demonstrated that small numbers of satellite cells can be generated from bone marrow grafts (LaBarge and Blau, 2002) and circulating $AC133^+$ cells (Torrente et al., 2004). MyoD mRNA determined in rat peripheral blood leukocytes suggests that a subset of circulating cells has the potential to act as myogenic precursor cells, even though the present study did not identify the exact

candidates.

Taken together, the results showed that cultured myoblasts expressed LTB_4 receptors BLT1 and BLT2, and BLT1 mediated the signaling function of LTB_4 to promote myoblast proliferation and differentiation. This suggested that LTB_4 acted as an inflammatory mediator to recruit leukocytes to the injury site to remove damaged tissue, and contributed to muscle regeneration by accelerating the proliferation of satellite cells and the differentiation of myoblasts.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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