BASAL GENE EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR-RELATED TRANSCRIPTIONAL FACTORS IN RAT SKELETAL MUSCLE DIFFERS BETWEEN SLOW AND FAST FIBER TYPES

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Abstract

Skeletal muscle is comprised of multiple fiber types. Slow-twitch oxidative muscle fibers have greater capillary density compared with fast-twitch glycolytic fibers of skeletal muscle. To gain insight into the molecular mechanism underlying the difference of capillary density, we investigated whether the basal gene expression of vascular endothelial growth factor(VEGF), a major angiogenesis-related factor, and its transcriptional factors(hypoxia-inducible factor-1 α , transforming growth factor- β 1, c-jun, and c-fos)differs between these two fiber types of rat skeletal muscle. The mRNA expression of VEGF and its transcriptional factors was significantly higher in slow type fiber of muscle(soleus muscle)compared with fast type fiber of muscle (plantaris and tibialis anterior muscles). These results suggest that the difference of basal gene expression of VEGF and its transcriptional factors between slow and fast fiber types of skeletal muscle may partly contribute to the difference in capillary density between these two fiber types.

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key word : capillary density, hypoxia-inducible factor-1 α , transforming growth factor- β 1, c-jun, c-fos

Introduction

Skeletal muscle is mainly composed of three fiber types, such as typeI(i.e., slow-twitch fiber), type-IIA, and typeIIB(i.e., fast-twitch fiber)¹). The metabolic and vascular properties differ between slow- and fast-twitch fibers of muscle¹). Slow-twitch fiber has high oxidation, while fast-twitch fiber has high glycolysis¹). The capillary density in slow-twitch fiber is greater than in fast-twitch fiber¹). Because oxygen transport conductance is positively related to the number of capillaries per muscle fiber, it is apparent that angiogenesis plays an important role in skeletal muscle function.

Vascular endothelial growth factor(VEGF)is a potent mitogen of endothelial cells and implicates in angiogenesis of animals and human²). The VEGF mRNA expression is regulated by transcriptional factors, such as hypoxia inducible factor(Hif)-1 α , transforming growth factor(TGF)- β 1, c-jun, and

c-fos^{3~6}). Recently, it has been reported that basal level of VEGF mRNA expression is higher in slow-twitch fiber compared with fast-twitch fiber of skeletal muscle⁷). However, the difference in basal gene expression of these VEGF-related transcriptional factors between slow-twitch fiber and fast-twitch fiber in the skeletal muscle is unclear.

Because VEGF participates in the homeostatic angiogenesis in the muscles, we hypothesized that the difference of gene expression of VEGF and its transcriptional factors between slow and fast fiber types of skeletal muscle participates in difference in capillary density between these two fiber types. The present study investigated whether the basal gene expression of VEGF and its transcriptional factors (Hif-1 α , TGF- β 1, c-jun, and c-fos)differs between slow-twitch and fast-twitch fibers of the skeletal muscle in rats.

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Methods

Animals. Five male 12-week-old Wistar rats were obtained from the Institute for Animal Reproduction (Ibaraki, Japan) and cared for according to the Guiding Principles for the Care and Use of Animals based on the Helsinki Declaration of 1964. The rats were anesthetized with diethyl ether. After anesthesia, soleus muscle (slow-type fiber) and plantaris and tibialis anterior muscles (both fast-type fiber) were rapidly excised and washed thoroughly with cold saline to remove contaminating blood and frozen in liquid nitrogen. The tissue samples were stored at -80°C for determination of VEGF mRNA expression by reverse transcription and real-time quantitative PCR analysis. In these muscles, the expressions of Hif-1 α , TGF- β 1, c-jun, and c-fos mRNAs were also determined by reverse transcription and real-time quantitative PCR analysis.

Reverse transcription and real-time quantitative PCR analysis. Total tissue RNA was isolated using Isogen (Nippon Gene; Toyama, Japan) as according to our previous paper⁸. Total tissue RNA ($2\text{ }\mu\text{g}$) was primed with $0.05\text{ }\mu\text{g}$ of oligo d(pT)₁₂₋₁₈ and reverse transcribed by omniscript reverse transcriptase using cDNA synthesis kit (Qiagen). The reaction was performed at 37°C for 60 min. Quantitative real-time PCR was used for measurement of mRNA expression (ABI-PRISMA 7700 Sequence Detector, Perkin-Elmer Applied Biosystems, CA, USA). The gene-specific primers and TaqMan (FAM) probes were synthesized from Primer Express v. 1.61 software (Perkin-Elmer). The sequences of the oligonucleotide were as follows:

VEGF forward : 5'-TGAGACCCTGGTGGACATCTT-3',

VEGF reverse : 5'-CACACAGGACGGCTTGAAGA-3',

VEGF probe : 5'-CCCCGATGAGATAGAGTAT-3',

Hif-1 α forward : 5'-CAACTGCCACCACTGATGAATC-3',

Hif-1 α reverse : 5'-GTAGAAGGTGGAGATGCAATCAGTAT-3',

Hif-1 α probe : 5'-TCCTTCGTCAGTCTTTT-3',

TGF- β 1 forward : 5'-CGGGAAGCAGTGCCAGAA-3',

TGF- β 1 reverse : 5'-TGCTCCACAGTTGACTTGAATCTC-3',

TGF- β 1 probe : 5'-CAGAGCTGCGCCTGCA-3',

c-fos forward : 5'-CTGGTGCAGCCCACTCT-3',

c-fos reverse : 5'-CTCCCGCTCTGGCGTAAG-3',

c-fos probe : 5'-CCCCATCGCAGACCAG-3',

c-jun forward : 5'-GAGCCAAGAAGCTCGGACCTT-3',

c-jun reverse : 5'-CCATTGCTGGACTGGATGATCAG-3',

c-jun probe : 5'-CTGCTCAAGCTGGCGTC-3',

GAPDH forward : 5'-GTGCCAAAAGGGTCATCATCTC-3',

GAPDH reverse : 5'-GGTTCACACCCATCACAACATG-3',

GAPDH probe : 5'-TTCCGCTGATGCCCC-3'.

Each PCR amplification was performed in triplicate, using the following profile: 1 cycle of 95°C 10 min and 40 cycle of 94°C for 15 sec and 60°C for 1 min. The expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used as an internal control. The quantitative values of VEGF, Hif-1 α , TGF- β 1, c-jun, and c-fos mRNA were normalized by that of GAPDH mRNA expression.

Statistics. Values are expressed as means $\pm$ SE. Statistical analysis among the soleus, plantaris, and tibialis anterior muscles was carried out by analysis of variance followed by Scheffé's F-test for multiple comparisons. $P < 0.05$ was accepted as significant.

Results

The mRNA expression of VEGF in the skeletal

Figure 1. Expression of vascular endothelial growth factor (VEGF) mRNA expression in the plantaris, tibialis anterior, and soleus muscles. The expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used as an internal control to normalize the gene expression data. Values are relative ratio of tibialis anterior and soleus to plantaris muscles. Open circles are mean value $\pm$ SE.

muscle was significantly higher in slow type fiber (soleus muscle) compared with fast type fiber (plantaris and tibialis anterior muscles) (Figure 1). The mRNA expressions of Hif-1 α , TGF- β 1, c-jun, and c-fos in the skeletal muscle were significantly higher in slow type fiber (soleus muscle) than in fast type fiber (plantaris and tibialis anterior muscles) (Figure 2A-D). There were no significant dif-

ferences in mRNA expression levels of VEGF, Hif-1 α , TGF- β 1, c-jun, and c-fos between plantaris and tibialis anterior muscles (Figure 1 and 2).

Discussion

The present study demonstrated that the basal level of gene expression of VEGF, a major angiogenesis-related factor, was higher in slow type

Figure 2. (A) Expression of hypoxia inducible factor-1 α (Hif-1 α), (B) transforming growth factor- β 1 (TGF- β 1), (C) c-jun, and (D) c-fos mRNA in the plantaris, tibialis anterior, and soleus muscles. The expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used as an internal control to normalize the gene expression data. Values are relative ratio of tibialis anterior and soleus to plantaris muscles. Open circles are mean value $\pm$ SE.

fiber than in fast type fiber of skeletal muscle. We also revealed that the basal level of gene expression of VEGF-related transcriptional factors (Hif-1 α , TGF- β 1, c-jun, and c-fos) in the skeletal muscle was higher in slow type fiber compared with in fast type fiber. It is generally accepted that the capillary density in slow-twitch fiber of skeletal muscle is greater than in fast-twitch fiber¹⁾. Therefore, the present findings suggest that the difference of basal gene expression of VEGF and its transcriptional factors between slow and fast fiber types of skeletal muscle may partly contribute to the difference in capillary density between these two fiber types.

VEGF plays a critical role in both physiological and pathological angiogenesis⁹⁾. The VEGF lacking mice was unable to survive by an impairment of vessel formation in the early embryo¹⁰⁾. Tang et al.¹¹⁾ reported that an inhibition of VEGF expression in the skeletal muscle induced the reduction of angiogenesis in muscles. Several studies have shown that intramuscular or arterial gene transfer of VEGF stimulated angiogenesis and resulted in improvement of hemodynamic deficit in the animal model of hind-limb ischemia^{12,13)}. Taken together, these observations suggest that VEGF participates in the regulation of the homeostatic angiogenesis in the skeletal muscle. The present study showed that VEGF gene expression in slow type fiber (capillary density is rich) was higher than in fast type fiber of skeletal muscle. Therefore, VEGF may be a responsible regulating factor for the muscle capillary maintenance in each slow and fast type fibers of skeletal muscle.

The VEGF mRNA expression is mainly regulated by multiple transcriptional factors, such as Hif-1 α , TGF- β 1, c-jun, and c-fos^{3~6)}. VEGF mRNA expression was markedly decreased by a deficiency of Hif-1 α in embryonic stem cell⁶⁾. The TGF- β 1 lacking (TGF- β 1^{-/-} and TGF- β 1^{+/-}) mice were died by a defect of vasculogenesis in the early embryo³⁾. The heterodimer of c-fos and c-jun, which is activated by c-jun N-terminal kinase (JNK), forms activate protein (AP)-1 and is considered to

play a key role in the transcriptional regulation of VEGF⁴⁾. JNK specific-inhibitor caused to an inhibition of VEGF production through JNK-depend signaling pathway⁴⁾. These previous studies suggest that the transcriptional factors (Hif-1 α , TGF- β 1, c-jun, and c-fos) regulate the transcription of VEGF gene through each different molecular mechanism. The present study revealed that the gene expression of VEGF-related transcriptional factors (Hif-1 α , TGF- β 1, c-jun, and c-fos) in slow type fiber of skeletal muscle was higher than that in fast type fiber of skeletal muscle. Therefore, although there are differences in the mechanisms underlying the transcriptional regulation of VEGF gene among transcription factors, it is considered that multiple transcription factors, i. e., Hif-1 α , TGF- β 1, c-jun, and c-fos, regulate VEGF gene expression, thereby contributing to the difference in capillary density between slow type fiber and fast type fiber of skeletal muscle.

In summary, the present study demonstrated that the basal level of gene expression of VEGF and its transcriptional factors, such as Hif-1 α , TGF- β 1, c-jun, and c-fos, differed between slow and fast fiber types of skeletal muscle. These results suggest that the difference of basal gene expression of VEGF and its transcriptional factors between slow and fast fiber types of skeletal muscle may partly contribute to the difference in capillary density between these two fiber types.

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