Study on the developmental expression of Lbx1 gene in longissimus dorsi of Mashen and Large White pigs

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Abstract

In the present study we investigated the developmental expression patterns of Lbx1 gene in skeletal muscle of different genetic profile pigs after birth. A total of 28 Mashen pigs and 28 Large White pigs from seven developmental stages of 1, 30, 60, 90, 120, 150, 180 d were used and the expression patterns in longissimus dorsi were studied by quantitative real time-polymerase chain reaction and western blot in this study. The results showed that the breed, age, and the interaction of breed and age significantly affected the gene expression in the developmental stages and to provide basic information for the migration of muscle precursor cells (Martin and Harland, 2006). In Lbx1 mutant mice, muscle precursor cells of limb delaminated from the ventral dermomyotome and failed to migrate laterally into the limb (Gross et al., 2000). In addition, Lbx1 was also involved in cell proliferation through the downregulation of p27 (Watanabe et al., 2006). In six month old pigs, Lbx1 was also detected in skeletal and cardiac muscles (Chao et al., 2011).

Mashen pig is a local breed in Shanxi Province and other regions of Northern China for thousands of years. Owing to the varied terrain, mountainous environment, natural economic and ecological conditions of Shanxi Province, the Mashen pig breed was selected for this study. The objective of this study was to characterise Lbx1 expression patterns in porcine skeletal muscle during different developmental stages and to provide basic information for further investigating its role in skeletal muscle development after birth.

Introduction

Lbx (ladybird-like), originated from a part of Nk homeobox cluster of metazoan, encodes transcription factors which can regulate lots of tissues development, including skeletal muscle, heart, central nervous system and sensory organs (Wotton et al., 2009). Lbx family contains 4 members, which are divergent in different vertebrates. Two members (Lbx1 and Lbx2) were found expressed in bony of vertebrates, and played an essential role during muscle and neural development (Pollard and Holland, 2000). Lbx2 was undetectable in chicken, but a novel homeobox gene Lbx3 was detected and expressed in prospective hypaxial myoblasts at cervical and limb level (Kanamoto et al., 2006).

Lbx1 was mainly expressed in the embryo, central nervous system and skeletal muscle, and was essential for the development of these tissues (Chen et al., 1999). During myogenesis in mammals and amphibians, Lbx1 was necessary for the migration of muscle precursor cells (Jagla et al., 1997; Schafer and Braun, 1999; Gross et al., 2000). Knockdown of Lbx1 was associated with a specific reduction of body wall muscles and hypoglossal muscles originating from the somites (Martin and Harland, 2006). In Lbx1 mutant mice, muscle precursor cells of limb delaminated from the ventral dermomyotome and failed to migrate laterally into the limb (Gross et al., 2000). In addition, Lbx1 was also involved in cell proliferation through the downregulation of myoD and p27 (Martin and Harland, 2006). In explanted somites in chicken, overexpression of Lbx1 and Pax3 could increase the cell proliferation dramatically (Mennerich and Braun, 2001). In activated satellite cells of adult mice, Lbx1 was also detected, which might suggest its roles in the differentiation of satellite cells in mature muscle fibres (Wotton et al., 2006). In six month old pigs, Lbx1 was also detected in skeletal and cardiac muscles (Chao et al., 2011).

Lbx1 gene on myogenesis were mainly focused on the embryo stage, and little data was reported after birth. The objective of this study was to characterise Lbx1 expression patterns in porcine skeletal muscle during different developmental stages and to provide basic information for further investigating its role in skeletal muscle development after birth.

Materials and methods

The use of animals and sample collection

All animal procedures were approved by the Code of Ethics of the World Medical Association (Declaration of Helsinki) for animal experiments (http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm). A total of 28 healthy Mashen and 28 healthy Large White pigs were selected from Datong Pig Breeding Farm (Shanxi, China) for this study. The piglets were weaned at four...
weeks old and the males were castrated at weaning for both Mashen and Large White. Four pigs (2 males and 2 females) of each breed were weighed and slaughtered at each of the seven development stages at 1, 30, 60, 90, 120, 150 and 180 days after birth, respectively. *Significance at the level of 0.05 is considered to be significant.*

**Results**

Large White and Mashen pigs possess different body weight and growth rate at different stages

The body weights of Large White and Mashen pigs at different stages were measured (Figure 1). Large White were more heavy than those of Mashen at each stage except for the birth weight (P<0.05). For Large White pig, the growth rate before weaning was 195.67 g/d, 541 g/d during 30 to 90, and above 1070.50 g/d during 90-150 d. For Mashen pigs, the growth rate was also lower before weaning and became faster during 30 to 60 d, with the growth rate of 410.33 g/d, then grew slower again with the growth rate of 180.33 g/d during 60 to 90 d, and afterwards, the growth rate was 583.22 g/d.

**Statistical analysis**

Lbx1 mRNA contents are different in Mashen and Large White *longissimus dorsi* muscle

Our data shows that Lbx1 mRNA relative expression was affected significantly by the effects of breed, age, and the interaction of breed and age (P<0.05) (Table 1). The developmental expression pattern of Lbx1 mRNA in *LD* muscle of both Large White and Mashen pigs is shown in Figure 2. The mRNA content in Large White was significantly higher than that of Mashen before 30 d, and lower after 30 d. In Mashen pigs, the Lbx1 mRNA contents kept a stable level before 120 d and there was no difference among these stages. After 120 d (with

![Figure 1. Body weights of Large White and Mashen pigs after birth (day 1, 30, 60, 90, 120, 150, and 180). Data are shown as mean±standard error of mean. *Significance at the same stage for Large White and Mashen pigs (P<0.05).*](image)

**Table 1. Analysis of variance table for Lbx1 mRNA relative expression.**

| Source of variation | Degrees of freedom | Sum of squares | F     | P     |
|---------------------|--------------------|---------------|-------|-------|
| B                   | 1                  | 3.6383        | 26.33 | 0.0001|
| DOA                 | 6                  | 38.8993       | 46.51 | 0.0001|
| B×DOA               | 6                  | 18.3110       | 22.68 | 0.0001|

B, Breed; DOA, day of age.
the increase of age), the mRNA showed an increase trend, and reached the highest value – which was greater than those at other stages – at 180 d (P<0.05). In Large White, the mRNA amounts were higher at 30 and 180 d than those at other stages (P<0.05), while was the least at 60 d.

Differential expression of Lbx1 protein in Mashen and Large White longissimus dorsi muscle

To further analyse Lbx1 protein expression at different developmental stages, western blot was performed. In accordance with the Lbx1 mRNA expression, the Lbx1 protein level was also affected significantly by the breed, age, and the interaction of breed and age (P<0.05) (Table 2).

The developmental expression pattern of Lbx1 protein in LD muscle is shown in Figure 3A and B. In Large White, the Lbx1 protein level showed an up-down-up-down trend; the expression was increased with the increase of age before 60 d, which was greater than those in Mashen pigs at the same age. After that, its content was decreased dramatically and reached the lowest level at 60 d, and then increased to reach the second expression peak at 90 d, and subsequently gradually decreased with the age. In Mashen pig, Lbx1 protein level showed an increase trend with the age increase before 90 d, then decreased to the lowest level at 120 d, and then increased gradually up to the expression peak at 180 d.

**Discussion**

In vertebrates, skeletal muscle is formed by the fusion of mononuclear precursor cells termed myoblasts. In adult skeletal muscle, some myoblasts remain undifferentiated as satellite cells. Satellite cells are located between the sarcolemma and the basement membrane of terminally differentiated muscle fibres (Campion et al., 1979; Bischoff and Heintz, 1994). The number of satellite cell was abundant when the animal was young and was decreased after aging. Normally, satellite cells are mitotically quiescent and replicate very slowly to self-renewal (Schultz, 1996; Decary et al., 1997). When muscles were damaged, these cells were activated and underwent multiple rounds of cell division, and then migrated to the damage sites, formed multinucleate myofibres either de novo or by fusion with preexisting muscle fibres (Seale and Rudnicki, 2000; Watanabe et al., 2007). In pig, the number of myofibres was fixed in postnatal growth of

![Figure 2. Developmental expression patterns of Lbx1 mRNA in longissimus dorsi muscle of Large White and Mashen pigs. Data are shown as mean±standard error of mean. 18S rRNA was used as reference gene. *Difference of mRNA relative expression of Lbx1 gene at the same age for Large White and Mashen pigs (P<0.05). Bars with no common letter within the same breed differ significantly (P<0.05).](image1)

![Figure 3. Developmental expression patterns of Lbx1 protein in longissimus dorsi muscle of Large White and Mashen pigs. A) Western blot pattern. Glyceraldehyde 3-phosphate dehydrogenase was used as reference protein. B) Relative abundance of Lbx1 proteins in longissimus dorsi of Large White and Mashen pigs at new birth, 30, 60, 90, 120, 150 and 180 days of age. Data are presented as mean±standard error. *Difference of Lbx1 relative expression at the same age between Large White and Mashen was significant at the level of 0.05. Bars with no common letter within the same breed differ significantly (P<0.05).](image2)

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Table 2. Analysis of variance table for Lbx1 protein relative expression.

| Source of variation | Degrees of freedom | Sum of squares | F       | P       |
|---------------------|--------------------|----------------|---------|---------|
| B                   | 1                  | 0.4556         | 27.75   | 0.0001  |
| DOA                 | 6                  | 15.6269        | 158.66  | 0.0001  |
| B×DOA              | 6                  | 40.8049        | 414.23  | 0.0001  |

B, Breed; DOA, day of age.

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skeletal muscle, and only the fibre size was increased (Swatland and Cassens, 1973; Swatland and Kieffer, 1974). Muscle hypertrophy depended on the increasing of myofibrils and of the nucleus which relayed on fusion of satellite cells (Campion et al., 1981).

Functionally, Lbx1 could induce myogenesis through guiding muscle precursor migration to the proper regions (Schafer and Braun 1999; Schmitteckert et al., 2011) and controlling myoblasts proliferation (Mennerich and Braun, 2001; Martin and Harland, 2006). Furthermore, Lbx1 played important roles in satellite cells differentiation and self-renew (Watanabe et al., 2007). Lbx1 gene was specifically expressed in central nervous system and muscles during embryogenesis in mouse (Jagla et al., 1995), and it was expressed in myoblasts that contributed to the body wall musculature (Martin and Harland, 2006). Previous studies demonstrated that in Meishan pigs, the Lbx1 gene was moderately expressed in muscle and heart, and weakly or hardly expressed in other organs at 120 days old (Chao et al., 2011).

In the current study, Lbx1 was detectable at seven post-natal stages, including 1, 30, 60, 90, 120, 150 and 180 d in Large White and Mashen pigs of LD muscle. In Large White, both the mRNA and protein amounts of Lbx1 were greater at birth and 30-d than those at other stages, which is consistent with previous reports (Chao et al., 2011).

In our study, all piglets were weaned at four weeks old. After weaning, the Lbx1 expression was decreased to the lowest level at 60 d, and then showed a sharp increase at 90-d following a gradually decrease to the lowest level again at 180 d in Large White. Lbx1 is mainly expressed in activated satellite cells, the fusion of satellite cells with myofibres and myofibrils increasing can result in myofibres hypertrophy after birth (Mesires and Doumit, 2002). The percentage of activated satellite cells was highest in 1 week after birth and was gradually decreased in 7 weeks age in pigs; between 7 to 21 weeks, the percentage of satellite cells slightly declined (Mesires and Doumit, 2002). Because of the decline of activated satellite cells, it was possible that Lbx1 expression was gradually decreased. In addition, the Lbx1 expression pattern in Large White also corresponded to its growth and development. The fast development of muscle during the first month is most likely attributed to the Lbx1 higher expression level in activated satellite cells.

Our data suggests that the Lbx1 expression pattern in Mashen pig is different from that in Large White, which might be due to the different genetic profiles between these two breeds. The Lbx1 mRNA expression in Mashen pig was increased from new birth to 180 d, with the peak appearing at 180 d. The protein content was also increased with age, except for 120 d, indicating that the percentage of activated satellite cells in Mashen skeletal muscle was higher. Regarding the expression pattern in these two pig breeds, Lbx1 expression in Large White was higher before weaning and lower after weaning than that in Mashen pig. The proliferation ability of muscle precursor in Large White is most likely greater than that in Mashen during embryogenesis, and this potential remains at a higher level after birth. Thus, the Large White grew faster than Mashen among whole stages. However, Mashen pig is a Chinese indigenous pig, one of its the biological characteristics is being very active. Previous studies show that exercise could induce satellite cell proliferation both in animal models (Li et al., 2006) and humans (Kadi et al., 2005). In addition, as a Chinese indigenous pig, the proliferative potential of satellite cells in Mashen pig was higher than in western commercial breed (Wang et al., 2012). The percentage of activated satellite cells in Mashen pig could be greater than in Large White at the same stage. As Lbx1 was mainly expressed in activated satellite cells after birth, so the expression content in Mashen pig was higher than in Large White after weaning. To demonstrate this, however, more studies need to be done in the future.

Conclusions

The expression patterns of Lbx1 mRNA and peptide showed that Lbx1 expression level is related to age and genetic profiles of Mashen and Large White pigs. Growth rate is associated with Lbx1 expression levels. Furthermore, Lbx1 was expressed in activated satellite cells and the differentiation of satellite cells results in muscle development after birth. We then hypothesise that Lbx1 may promote skeletal muscle development by activating satellite cell after birth.

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