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Expression Changes of Caveolin Family Genes in Longissimus Dorsi Muscle and Back Subcutaneous Fat of Two Pig Breeds

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Abstract: Caveolins have important roles in the organization of detergent-insoluble lipid rafts, trafficking of cholesterol and anchoring of signaling molecules. In this study, the expression patterns of *Caveolin-1* (*Cav-1*), *Caveolin-2* (*Cav-2*) and *Caveolin 3* (*Cav-3*) genes were investigated in the longissimus dorsi muscle and back subcutaneous fat of Berkshire and Yacha pigs at the age of 6 months using quantitative real-time PCR. The results showed that Cav-1 and -2 mRNA were abundantly expressed in back subcutaneous fat. The expression patterns of Cav-1 and -2 were similar in the two pig breeds. The expression levels in the back subcutaneous fat were significantly higher in Yacha pigs than in Berkshire pigs ($p<0.01$). Cav-3 was expressed predominantly in the longissimus dorsi muscle and the Berkshire pig had higher Cav-3 mRNA levels compared with the Yacha pig ($p<0.01$). These results indicate that the mRNAs of Cav family genes exhibit specific expression changes between Berkshire and Yacha pigs. The data contributes to the elucidation of the relationship between meat quality and Cav family genes expression and provides a basis for further study on the mutual regulation mechanism of Cav family genes.

Key words: Longissimus dorsi muscle, back subcutaneous fat, Caveolin, pig, quantitative real-time PCR, China

INTRODUCTION

Caveolae are small invaginations of the plasma membrane known to play important roles in signal transduction, membrane trafficking and cholesterol homeostasis (Anderson, 1998; Fujimoto *et al*., 1998; Okamoto *et al*., 1998; Ikonen *et al*., 2004; Krajewska and Maslowska, 2004). Caveolins are essential for the above functions and form the structural framework of the caveolae (Cohen *et al*., 2004). The caveolin family has three members: Caveolin-1 (Cav-1), Caveolin-2 (Cav-2) and Caveolin-3 (Cav-3) (Anderson, 1998). Cav-1 and -2 are usually co-expressed and have a relatively ubiquitous distribution pattern in most differentiated cell types. They have important functions in lipid traffic, membrane traffic and signal transduction (Cohen *et al*., 2003; Gargalovic and Dory, 2003; Quest *et al*., 2004). The expression of Cav-3 is limited to cells of the myoblast lineage including cardiac, skeletal and smooth muscle cell types (Song *et al*., 1996). Some studies have shown that Cav-3 is involved in the formation of the transverse tubule system in developing muscle and it has been thought to play a key role in myocyte-specific functions (Parton *et al*., 1997; Galbiati *et al*., 2001; Capozza *et al*., 2005).

Sus scrofa (i.e., pig or swine) is an agriculturally important animal. Fat and muscle have been research foci because they represent economically important parts of the pig's carcass and because of their contributions to improving meat quality. Berkshire pigs, a typical lean-type western breed have been intensively selected over the past two decades for rapid, large and efficient accumulation of muscle. On the other hand, Yacha pigs, a typical fatty-type indigenous Chinese breed have lower growth rates and lower lean meat content but better meat quality than conventional western pig breeds. Caveolins have multiple functions in fat metabolism, gluconeogenesis and muscle development therefore, they are considered as very important candidate genes for improving meat quality. Thus, it is important to investigate the expression patterns of caveolins in the adipose and muscle tissues of pigs.

In this study, the researchers investigated the expression patterns of Caveolin family genes in the longissimus dorsi muscle and back subcutaneous fat of Berkshire and Yacha pigs at 6 months old (180 days).

MATERIALS AND METHODS

Animal and sample collection: Twelve pigs (Six Berkshire and six Yacha pigs, three female and three male of each breed) were used in this study. The animals were reared in compliance with national regulations for the humane care and use of animals in research. The animals were allowed

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Table 1: Primers used for q-PCR

Genes symbol	GenBank ID	Primer sequence (5'-3')	Amplicon length (bp)	Temp (°C)
Cav-1	NM_214438	F: ACAAGCCCAACAACAAG R: CAGACAGCAAACGGTAA	240	56
Cav-2	NM_001123091	F: TAAAGACCTGCCTAATGG R: AGTCATGGCTCAGTTGC	132	54
Cav-3	NM_001037149	F: GCATCAGCCATATCTACTCACT R: CACTTCTTTCCGCAGCAT	107	58
*ACTB**	DQ178122	F: TCTGGCACCACACCTTCT R: TGATCTGGGTCATCTTCTCAC	114	60
*TBP**	DQ178129	F: GATGGACGTTCGGTTTAGG R: AGCAGCACAGTACGAGCAA	124	60
*TOP2B**	AF222921	F: AACTGGATGATGCTAATGATGCT R: TGGAAAAACTCCGTATCTGTCTC	137	60

access to feed and water *ad libitum* under normal conditions and were humanely sacrificed as necessary, to minimize suffering. The samples were collected immediately after sacrifice. The longissimus dorsi muscle and back subcutaneous fat near the last 3rd or 4th rib were carefully and manually dissected, respectively from each of the 12 cleaved pigs. All samples were immediately frozen in liquid nitrogen and stored at -80°C until further use.

Muscle and adipose measurements: The longissimus dorsi muscle and back subcutaneous fat samples were sliced at a thickness of 8 μm using a KD-2508 rotary microtome (Kedee, Zhejiang and China) following the frozen section procedure which was described by Wilson (1905) and stained with Hematoxylin and Eosin (H and E). The myofiber Cross-Sectional Area (CSA) and adipocyte volume were counted for an average of 100 fibers and adipocytes in randomly selected fields using a BK5000 fluorescence microscope (Optec, Chongqing and China) and Motic Images Adwanced 3.2 software.

Total RNA extraction and cDNA synthesis: Total RNA was extracted using the mirVana kit (Ambion, Austin, USA) from the 24 tissue samples following the manufacturer's protocol. The total RNA quantity and purity was analyzed using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, USA) at 260/280 nm (Ratio>2.0) and by 1% denaturing agarose gels containing formaldehyde.

Equal amounts of RNA sample were reverse transcribed to cDNA templates using the oligo (dT) primer and random primer provided in the RT kit (TaKaRa, Dalian, China) for RT-PCR following the manufacturer's recommendations.

Q-PCR assays: Q-PCR was performed using the SYBR Green PCR kit (TaKaRa) on a CFX96 real-time PCR detection system (Bio-Rad, Hercules, USA). Each reaction comprised 12.5 μL of SYBR Green q-PCR Super Mix, 2 μL of cDNA, 10 pmol of each primer and RNA-free water (Ambion) to a total volume of 25 μL. The real-time PCR program started with 30 sec of denaturation at 95°C, followed by 40 cycles of 5 sec of denaturation at 95°C and 10 sec of annealing/elongation at the annealing Temperature (Tm) for each specific primer (Table 1) during which time, fluorescence was measured. The specific primers for the three target sequences were designed using Primer 5 software. The specific PCR products were confirmed by melting curve analysis; this allowed the verification of the presence of one gene-specific peak and the absence of primer dimers. All measurements contained a negative control (No cDNA template) and each RNA sample was analyzed in triplicate. Each objective gene was normalized to *ACTB*, *TBP* and *TOP2B*. Normalized Factors (NF) of internal control genes and relative quantities of objective genes were analyzed using the geNorm software (Vandesompele *et al*., 2002). Statistical analyses were performed by the SAS 9.1 package.

RESULTS AND DISCUSSION

As shown in Fig. 1, Berkshire pigs exhibited a higher CSA than Yacha pigs at 6 months old. However, Yacha pigs had a higher adipocyte volume than Berkshire pigs. These results correspond to their distinct breeding characteristics and reflect the fact that the meat quality of Yacha pigs is better than Berkshire pigs.

Expression of caveolin family genes mRNA was assessed by RT-PCR analyses in the back subcutaneous fat and longissimus dorsi muscle tissues from the two pig breeds. The study showed that Cav-1 and -2 mRNA were abundantly expressed in back subcutaneous fat and that the expression patterns were similar in the two pig breeds (Fig. 2a, b). In detail, back subcutaneous fat had higher Cav-1 and -2 expression levels than longissimus dorsi muscle in both pig breeds. In the back subcutaneous fat, the Cav-1 and -2 expression levels were significantly higher in Yacha pigs than in Berkshire pigs ($p<0.01$) whereas there was no significant difference of Cav-1 mRNA in the longissimus dorsi muscle between two pig breeds ($p>0.05$). Moreover, the researchers also found

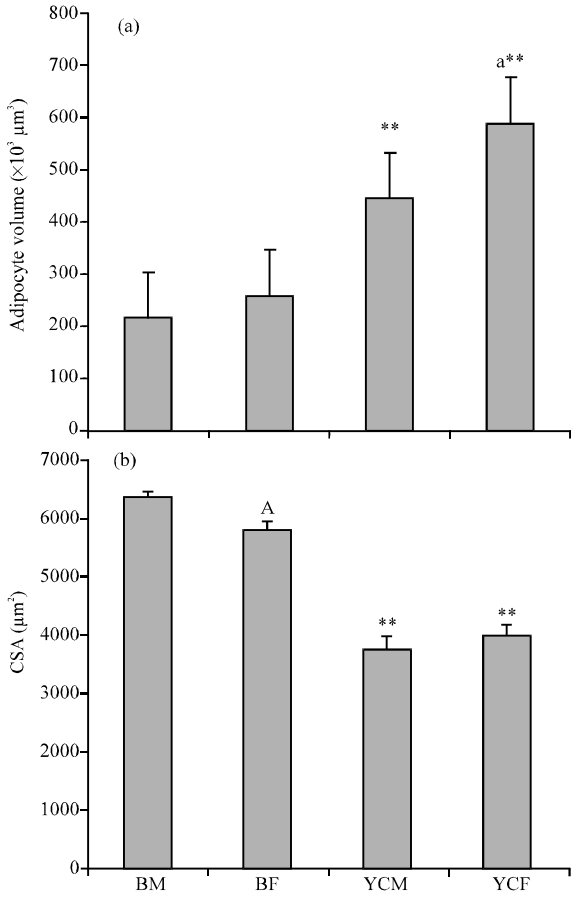

Fig. 1: a) Changes of CSA in two pig breeds and b) Changes of adipocyte volume in two pig breeds. Symbol **indicates a significant difference ($p<0.01$) between two breeds of the same gender; a different letter indicates significant difference at the 5% (Small letter) or 1% (Capital letter) levels between genders of the same breed. BM = Berkshire male pig; BF = Berkshire Female pig; YCM = Yacha Male pig; YCF = Yacha Female pig

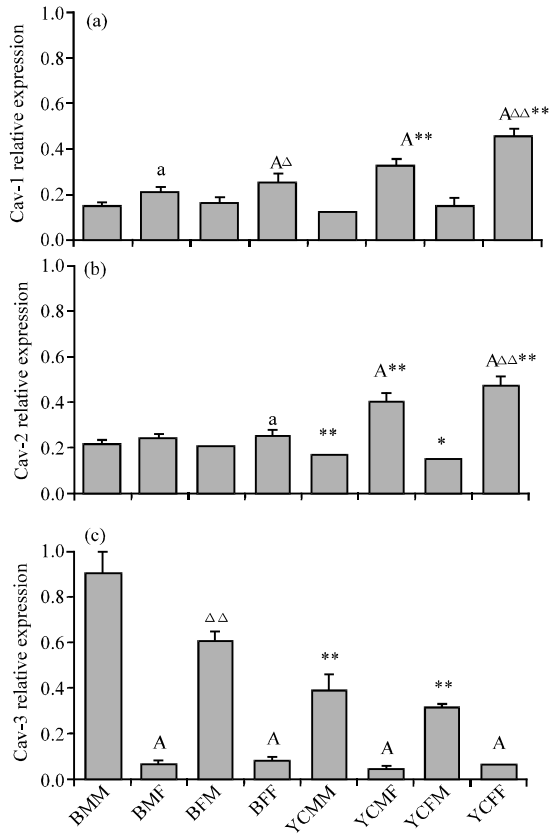

Fig. 2: a) Expression patterns of Cav-1 mRNA in longissimus dorsi muscle and back subcutaneous fat for the two pig breeds; b) expression patterns of Cav-2 mRNA in longissimus dorsi muscle and back subcutaneous fat for the two pig breeds and c) expression patterns of Cav-3 mRNA in longissimus dorsi muscle and back subcutaneous fat for two pig breeds. A different letter indicates significant difference at the 5% (Small letter) or 1% (Capital letter) levels between the two tissues of the same breed and gender. Symbol * or ** indicates significant difference at the 5 or 1% level between Berkshire and Yacha pigs of the same gender and tissue. Symbol ΔΔ or Δ indicates significant difference at the 1 or 5% level between the genders of the same breed and tissue. BMM = Muscle of Berkshire Male pig; BMF = Fat of Berkshire Male pig; BFM = Muscle of Berkshire female pig; BFF = Fat of Berkshire female pig; YCMM = Muscle of Yacha Male pig; YCMF = Fat of Yacha male pig; YCFM = Muscle of Yacha Female pig; YCFF = Fat of Yacha Female pig

that the Cav-1 expression level was significantly higher in the back subcutaneous fat of female pigs than in the back subcutaneous fat of male pigs in both Yacha pigs and Berkshire pigs (Yacha pig: $p<0.01$; Berkshire pig: $p<0.05$). The same result was found for Cav-2 only in Yacha pigs ($p<0.01$). As shown in Fig. 2c, Cav-3 was expressed predominantly in the longissimus dorsi muscle and the Berkshire pigs had higher Cav-3 mRNA levels than the Yacha pigs ($p<0.01$). Moreover, the expression of Cav-3 mRNA was significantly higher in Berkshire male pigs than in Berkshire female pigs ($p<0.01$). However in back subcutaneous fat tissue, Cav-3 mRNA levels were very low and no significant differences were observed between the two pig breeds. Previous studies have shown that Cav-1 and -2 are expressed in many cell types with especially high levels in endothelial cells and adipocytes

while Cav-3 is exclusively expressed in skeletal and cardiac muscle cells (Scherer *et al*., 1996; Tang *et al*., 1996; Singh *et al*., 2003). The study showed that the expression of Cav-1 and -2 in back subcutaneous fats of Chinese indigenous pigs (Yacha pig) were higher than that in the introduced pigs (Berkshire pig), accordingly the adipocyte volume of Yacha pig was higher than the Berkshire pig. These results indicated that Cav-1 and -2 could be involved in the regulation of adipocyte development and meat quality which is affected by fatty deposition. The results also validated the findings of previous researchers who reported that Cav-1 and -2 can be directed to lipid droplets and have important functions on lipid transport and glucose metabolism (Fujimoto *et al*., 2001; Razani *et al*., 2002; Pol *et al*., 2004; Blouin *et al*., 2008; Gonzalez-Munoz *et al*., 2009). By contrast in the longissimus dorsi muscle, Berkshire pigs had higher Cav-3 mRNA level and CSA than Yacha pigs. This is consistent with the findings of Zhu *et al*. (2006) who reported that Cav-3 expression level was higher in longissimus dorsi muscle of duroc pigs (Introduced pigs) than in Tongcheng pigs (Chinese indigenous pigs). This result indicates that porcine Cav-3 may play a positive role in muscle fiber development. This theory was supported by a study on Zebrafish embryos where Cav-3 knock-down was found to produce defects in myoblasts fusion as well as myofibrils and membrane systems disorganization (Nixon *et al*., 2005).

Galbiati *et al*. (1999) also reported that myotube formation was inhibited by antisense-induced down-regulation of Cav-3 in C2C12 differentiating myoblasts. Surprisingly, the researchers found that Cav-3 which is usually regarded as muscle-specific is expressed in back subcutaneous fat. This could be because of the increased sensitivity of qRT-PCR and further research should be performed.

CONCLUSION

The study showed that there were distinct expression patterns of Cav family genes between two pig breeds that differ in meat quality and growth rate. These findings suggest that the distinct differences in fat-deposition and muscle development ability between the two breeds closely correlate with the expression changes of these genes.

RECOMMENDATIONS

Furthermore, Cav-1, -2 and -3 could be regarded as important candidate genes that affect meat quality. The researchers envisage that this study will lead to a better understanding of the mechanism of caveolae signaling in muscle and adipose tissue.

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