The expression analysis of COL1A1, COL1A2 and procollagen type I in pig tissues

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***Abstract*—Korea's propensity to consume pork can be defined as the preference for the pork having parts with tenderness and texture due to our culture of roast. In this study, we've focused on the intramuscular fat and texture, important elements in the parts to be roasted and observed their relationship with meat quality of collagen type I that makes up 90% of collagen family that plays an important role in many connective tissues. We obtained collagen type I α-1 gene (COL1A1) and collagen type I α-2 gene (COL1A2) that produce collagen type I from porcine cDNA library. Then we proceeded with quantitative real-time PCR and immunoblotting analysis to understand what expression patterns two genes show by species, growth stages and tissues. By looking at real-time PCR results, we've observed that higher expressions were found in both tenderloin and backfat for growth stage 110kg compared to other growth stages. By comparing tenderloin and backfat, much higher expression level was found in backfat. As to different expression level between species, *Sancheong Berkshire* showed higher figure in tenderloin while *Yorkshire* showed higher figure in backfat ($p<0.01$ or $p<0.05$). Same patterns were found in Immunoblotting results just as in real-time PCR. Based on these results, we can infer that collagen type I is related with growth and that high expression level in fat tissues is related with meat quality. In addition, the fact that *Sancheong Berkshire* showed higher expression level in tenderloin compared to *Yorkshire* can be an evidence of the superior meat quality of *Sancheong Berkshire* as known in Korea. As we cannot be aware of the relationship between intramuscular fat, texture or meat quality just by expression level, we conclude that more in-depth study would be needed in future in order to understand the relationship between these genes and meat quality.**

***Index Terms*—Collagen type I; cDNA chip; Pig; meat quality**

I. INTRODUCTION

Koreans have been long been accustomed to eating roast pork thus there's preference for pork parts with tenderness, flavor and meat soluble as to appropriate intramuscular fat and texture. For this reason, Koreans enjoy black pigs that have higher level of intramuscular fat compared to other species (Hodgson et al., 1992; Eikelenboom et al., 1996).

We've initiated with molecular biology studies related to intramuscular fat and texture, among key elements of meat quality. Of those, we've made research on collagen type I that shows high expression level in fat compared to other tissues. Collagen is one of extra cellular matrix proteins and is a very important protein making up the structures of various organs including lung, skin, bone and blood vessels (Ghislaine et al., 2002; Zuping et al., 2005). Also, collagen's strong adhesiveness takes part in linking process in between cells and its high water holding capacity preserves the gene of hydrated membrane that protects cell membranes, thus providing flexibility of bone tissues (Johnstone I.L., 2000; Kivirikko K.I., 1992 and 1998).

Among them, collagen type I is the most abundant of the whole collagen family and makes up 70% of the bone's component as well as playing an important role in cartilage, brain, muscle, skin, ligament, cornea and many niche connective tissues (Tapio et al., 2004; Gelse et al., 2003). Procollagen type Ⅰ, a precursor of collagen type Ⅰ, forms heterotrimer with 2 identical α-1 chains and 1 α-2 chain thanks to two different genes, namely COL1A1 and COL1A2. It thus makes up triple helix with 3 polypeptide chains and also acts as collagen type I by proteolysis (Carine., 2006 and Pritam et al., 2005). Human COL1A1 and COL1A2 are located at 17q21.31-q22 and 7q21.3–q22.1 respectively and is are synthesized with procollagen molecules formed of N, C-terminal propeptides that are eliminated with site-specific endopeptidase (Raymond., 1997). Study on variation of COL1A1 genes among Collagen type I has been done many times before and there have been reports that this diminishes bone's mass and is a reason behind osteogenesis imperfecta (Tracy et al. 2006). There have also been reports that variation in No.23 exon in COL1A1 gene can cause cancer in human skin (Saeki et al., 2006). In the case of mouse, there have also been reports that variation in collagen type I is a reason behind osteogenesis imperfecta (Carine et al., 2006) and that collagen type I content decrease in muscular tissues of mouse with lessened physical

activity (Han et al., 1999). Among many studies regarding Collagen type Ⅰ, studies with pigs show reports that increase in collagen type I helps specialization of bones (Fuerst et al., 2004) Also, comparison between fetus with higher weight and lesser weight in the same mother led to the report that contents of intramuscular fat and collagen type I were higher in muscle of fetus with lesser weight (Karunaratne et al., 2005).

We have thought that strong adhesive strength, one of collagen's characteristics, takes part in linking of cells thus having relation with contents of pork texture and intramuscular fat. For this reason, although there haven't been any reports yet, we have confirmed gene expression patterns of genes (COL1A1, COL1A2) forming Procollagen type Ⅰ, precursor to collagen type Ⅰ.

II. MATERIALS AND METHODS

Animals

Sancheong Berkshire and *Yorkshire* (from Sungchuk Farm) female pigs whose body weight reached 60, 80, and 110 kg respectively 3 piglets were butchered, and their tissues were immediately taken, soaked in liquid nitrogen, and kept in a freezer at -80°C until RNA isolation

Total RNA extraction and Real-time PCR

Total RNA was extracted with TRIzol reagent according to the manufacturer's protocol (Life Technologies, Invitrogen). Trizol reagent (2 ml) was added to 0.1-0.2 g of tissue, ground and the concentration of total RNA was measured by absorbance, then confirmed by electrophoresis in 1.5% formamide gel. After quantification of total RNA by spectrophotometer (Nano Drop, USA), cDNA dilutions of 100, 10, 1 ng, and 100 pg were prepared to create a quantitative reference standard with Taqman Universal PCR Master Mix and SYBR Green PCR Master Mix (Applied Biosystems, USA). To obtain the maximum specificity in amplification, the primers were designed using the software Primer Express 3.0 (Applied Biosystems, USA). Primer sequences for GAPDH (Acc. No. AF017079) was reported in pig. Primer sequences for specific probe forward (F) and reverse(R) primers (Table2). Real-time PCR was performed on cDNA generated by the reverse transcriptase reaction using the sequence detector 7500 ABI PRISM (Applied Biosystems). Forty cycles were performed at the following temperatures: 95°C for 10 min, then 95°C for 30 s (Taqman) and 95°C for 10 min, then 95°C for 30 s and 60°C for 1 min (SYBR Green).

Analysis pro collagen type 1 expression by immunoblotting

After separation the lysates on 15% SDS-PAGE gels, the proteins were transferred to PVDF membranes. The membranes were saturated with 5% non-fat dry milk in TBS/Tween overnight at 4°C and, after washing with TBS/Tween, probed with a procollagen type 1 pAb (abcam, USA, 1:3,000 dilution in TBS/Tween with 5% BSA) for 3 h at room temperature. Then the blots were washed three times with TBS/Tween and incubated with a goat anti-rabbit secondary antibody conjugated to HRP (abcam, USA, 1:3,000 dilutions in TBS/Tween). The washed blots were treated with ECL reagents (GE Healthcare, UK) according to the manufacturer's instructions, and the bands were visualized luminographically on X-ray films (Kodak, USA).

III. RESULTS AND DISCUSSION

We were able to obtain collagen family genes that showed higher expressions in backfat than in tenderloins. Among them, COL1A1 and COL1A2 genes showed highest expression level of 94 times in backfat while collagen type Ⅴα-2 gene(COL5A2) and collagen type Ⅵ α-1 gene(COL6A1) showed expression level of 90 and 88 times respectively by using DNA chip analysis .

After producing inner primer (Table2) of COL1A1 and COL1A2 clone selected from DNA chip analysis in the end part of DNA sequence and confirming its full sequence, it was learned that COL1A1 was composed of 290 amino acids of 1,122bp (Figure 1 A) and that COL1A2 was composed of 198 amino acids of 931bp (Figure 1 B). Figure 2 alignments of the deduced in COL1A1 and COL1A2 gene amino acid DNA sequence of Homo sapiens, Bos Taurus, Mus musculus and the selected clone. As a result, the selected COL1A1 showed 96% similarity with Homo sapiens (genbank accession no. Z74615) and 95%, 90% similarities with Bos Taurus (genbank accession no. NP_001029211) and Mus musculus (genbank accession no. NP_031768) respectively (Figure 2 A). Also, COL1A2 showed 93% similarity with Homo sapiens (genbank accession no. NP_000080) and 93%, 85% similarities with Bos taurus(genbank accession no. BAA25171) and Mus musculus(genbank accession no. NP_031769) respectively (Figure 2 B).

The expression levels of COL1A1 and COL1A2 were determined with the tenderloin and backfat at each growth stage (weighing 60, 80, and 110 kg) obtained from *Sancheong Berkshire* and *Yorkshire* pigs by real-time PCR, as seen in Figure 3, where higher expression level was shown in backfat rather than in tenderloin. As to gene expression level difference between species, *Sancheong Berkshire* showed high expression level in tenderloin while *Yorkshire* showed high expression level in backfat (Figure 3 A, B).

Procollagen type Ⅰ's protein expression pattern produced by COL1A1 and COL1A2 can be known through immunoblotting. Procollagen type I protein was found substantially in both tenderloin and backfat tissues at 110kg, and higher expression level was shown in backfat rather than in tenderloin. As to gene expression level between species, *Sancheong Berkshire* showed high expression level in tenderloin while *Yorkshire* showed high expression level in backfat, just as in the case of mRNA (Figure 4).

IV. CONCLUSION

As Koreans have a penchant for consuming roast pork, it is thought that tenderness and texture at the moment of roasting pork are important elements among meat quality factors. Intramuscular fat content is closely related to these meat quality tendencies (Shourthose and Harris, 1990; Eikelenboom et al., 1996), and is also reported to have inverse correlation with drip loss (Lee et al., 1999). As *Sancheong Berkshire* has higher intramuscular fat content compared to other species, they have low shear force and hardness while showing high level of softness (Hodgson et al., 1992; Eikelenboom et al., 1996). These qualities make black pigs more in demand in Korea. Therefore, we have been observing gene expression pattern in fat tissues that are thought to be largely influential to meat quality and have consequently confirmed gene expression pattern using cDNA chip.

It has been observed that in a cDNA chip made up of 4,434 ESTs, all 62 clones, as collagen family, showed as much as 90 times higher expression level in backfat compared to tenderloin. With this result, we can infer collagen family's relationship with meat quality in backfat. Among these, we've cloned COL1A1 and COL1A2 genes that are collagen type I genes with highest expression level, confirmed their sequence (Figure 1) and reconfirmed mRNA and protein expression patterns through quantitative real-time PCR(figure 2 and 3) and immunoblotting(figure 4).

Collagen is a protein determining structure in almost all tissues and is reported to be influential to adhesiveness, water holding capacity and bone tissue's flexibility (Johnston, 2000; Kivirikko, 1992 and 1998). As seen from collagen's function in these studies, high expression level of collagen type Ⅰ's mRNA and protein in fat tissues in short let us infer its relation with meat quality in pork. In particular, as fat tissues have influence on meat's softness, collagen's high expression level is thought to be related with softness and texture. In addition, *Sancheong Berkshire*'s higher collagen type Ⅰ expression level ($p<0.05$), which shows high expression in backfat tissues, than *Yorkshire* in tenderloin can infer to substantial accumulation of intramuscular fat as well as conformity with ongoing reports that *Sancheong Berkshire* boasts of fine texture.

In terms of growth stage, it can be confirmed that regardless of species, the heavier the weight, the more collagen's content level thus showing highest expression level at 110kg that has the finest meat quality ($p<0.05$). While it's not clear which physiological mechanism this is, it can be inferred that collagen plays a vital role for meat's texture or growth in fat tissues. Based on Karunaratne's research results (2005), it has already been reported of a positive relationship between intramuscular fat content and collagen type I in pigs. This is an evidence to have more trust in the study's results.

So far, the research results concerning relationship between meat quality and collagen are considered inadequate. Collagen type I are mainly known to cause various diseases such as abnormality in human/animal bones or skin diseases when there's variation of 2 genes(COL1A1 and COL1A2) in case of human(Tracy et al., 2006; Saeki et al., 2006; Carine et al., 2006). There has been report that collagen type I helps bone's specialization even in pigs (Fuerest et al., 2004). Also, by going over study results using mouse, there's report that those mouse with decrease physical activity also saw their collagen type I content in muscles decrease (Han et al., 1989). In general, seeing that increased physical activity brings more muscular fiber which in turn enhances meat texture, we can infer that collagen type I content can indirectly influence on the meat quality.

We cannot yet conclude of any direct influence the collagen type I genes and proteins within pigs have on meat quality characteristics merely based on the high concentration of collagen type I genes and proteins in fat tissues. In future, we'll carry on research on collagen type I related surrounding factors and other mechanisms in order to confirm the link between meat quality and collagen type I with the aim of uncovering a more precise correlation.

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