Proteomics Comparison of *Longissimus* Muscle between Hanwoo and Holstein Cattle

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Abstract

This study was conducted to compare proteins expressed in *M. longissimus* from Hanwoo and Holstein steers immediately after slaughter. Two-dimensional electrophoresis (2DE)/LC-MS/MS analysis revealed that the total number of detectable protein spots from *longissimus* muscle tissues was slightly higher in Hanwoo (575 ± 65) than Holstein (534 ± 13) steers, but that these numbers were not statistically significant due to large variation between replicates. A total of twelve protein spots did not match between sample groups, eight of which were expressed in the Hanwoo sample and four that were expressed in the Holstein sample. The protein spots detected in the Hanwoo sample included smooth muscle and non-muscle myosin alkali light chain 6B isomers, α B crystallin isomers, hemoglobin β -A chains, slow myosin heavy chains, and slow skeletal muscle troponin T chains. Collectively, these proteins are a class of slow-twitch muscle fiber and mirror that Hanwoo muscle tissue sampled for the current study contained more slow-twitch muscle fibers than Holstein one. Conversely, proteins detected from the Holstein sample included ankyrin repeat domain 2 and creatin kinase isomers. Given that creatin kinase isomers are related to the fast-twitch muscle, these results likely indicate that Holstein muscle tissue sampled for the current study contained more fast-twitch muscle fibers than Hanwoo beef.

Key words: Hanwoo, Holstein, muscle proteome, meat quality, slow-twitch muscle

Introduction

The main sources of beef in Korea come from Hanwoo. Hanwoo, the Korean native cattle, have been bred for the production of high meat quality such as marbling, softness and flavor. Consequently, Hanwoo is the most prominent beef breed of Korea and famous for its ability to produce high-quality meat. Holstein cattle are known for their excellent milk output capabilities and the male Holstein has been sold as a source of domestic beef, although the meat quality is inferior to Hanwoo beef (Kim *et al.*, 1993; Oh, 1990). As the meat quality of the Hanwoo is better than that of Holstein, Hanwoo beef consumption is increasing in spite of the higher price. Despite the big gap in the meat qualities between Hanwoo and Holstein cattle, molecular characteristics underlying the differences in

meat qualities between Hanwoo and Holstein are not well known.

Recently, there have been many proteomic studies involving the analysis of cellular proteins using diverse technologies, such as a two-dimensional electrophoresis (2DE), LC-MS/MS analysis, and NCBI database searches have been reported (Hwang *et al.*, 2005; Jung *et al.*, 2005; Kim *et al.*, 2006; Ohsaki *et al.*, 2007). The 2DE can separate all proteins contained in a sample by its specific isoelectric point and molecular weights. Comparing 2DE gel pattern between Hanwoo and Holstein muscle samples, candidate proteins that contribute to the different characteristics of meat qualities could be found (Kim *et al.*, 2008; Ohsaki *et al.*, 2007). These candidate proteins can be identified using LC-MS/MS analysis and information in the NCBI database (Ohsaki *et al.*, 2007). Therefore, our study was performed to compare protein profile between Hanwoo and Holstein *longissimus* muscle.

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Materials and Methods

Animals

In order to compare protein profiles between Hanwoo and Holstein beef, *M. longissimus dorsi* were taken immediately after slaughter from each five steers at 32 (or 34) mon old from a slaughterhouse. All samples were stored at -80°C prior to sample preparation for 2DE.

Two-dimension electrophoresis and staining

2DE was performed as described by Hwang *et al.* (2004), with minor modifications. Half gram of muscle tissue was homogenized using a homogenizer (Ultra-Turrax T25, USA) for 30 s in 1 mL of lysis buffer, containing 7 M urea, 2 M thiourea, 2% CHAPS, 1% dithiothreitol (DTT), and 0.8% Pharmalyte pH 3-10 linear, a procedure that was repeated 3 times. The homogenate was vortexed for 1 min and incubated at room temperature for 30 min with rotational shaking. After shaking, the homogenate was then centrifuged at 40,000 *g* for 30 min at room temperature, and the supernatant was collected. Protein concentration of the supernatant was determined with a PlusOne 2-D Quant kit (GE healthcare, USA) according to the manufacturer's instructions.

Isoelectric focusing (IEF) was performed with a Protean IEF Cell (Bio-Rad, USA) using 24 cm linear range of pH 3-10 IPG (immobilized pH gradient) strip gels (Bio-Rad, USA). Muscle protein (0.8 mg) samples in the supernatant were applied to the strip holder supplemented with rehydration buffer (450 μ L/strip), containing 8 M urea, 0.2% DTT, and 0.2% Pharmalyte pH 3-10 linear. The strip gels were then rehydrated for 12 h. IEF was carried out initially at 250 V for 30 min, increasing the voltage to 10,000 V linearly over 1 h, kept at 10,000 V for 8 h, and then finally at 500 V for 30 min at 50 mA/strip for 70 kVh at 20°C. After IEF separation, the strips were equilibrated in equilibrium buffer A (50 mM Tris-HCl, pH 8.8, 6 M urea, 30% glycerol, 2% SDS, 0.002% bromophenol blue, and 1% DTT) for 15 min, followed by an additional equilibrium for 15 min with equilibrium buffer B (50 mM Tris-HCl, pH 8.8, 6 M urea, 30% glycerol, 2% SDS, 0.002% bromophenol blue, and 4.8% iodoacetamide). The second dimension electrophoresis was performed with 12% acrylamide (37.5:1 ratio of acrylamide to bisacrylamide) using Protean Plus Dodeca Cell (Bio-Rad, USA) at 120 V for 30 min and 200 V until the line of bromophenol blue reached the bottom of the gel. Gels were stained by 0.1% colloidal Coomassie Brilliant Blue G-250 (CBBG) in 2% phosphoric acid, 10% ammonium

sulphate, and 20% methanol for 48 h (Westermeier and Naven, 2002), and were digitalized by an imaging system (VersaDoc MP 5000 system, Bio-Rad, USA).

Image analysis and spot identification

The gels were analyzed using 2DE image analysis software (PDQuest, Bio-Rad, USA) according to the manufacturer's instructions. Spots appearing in only breed of cattle in optical density were accepted as analytical spots and were excised using an automated spot cutter (Proteome Works, Bio-Rad, USA) as a circular plug 1.5 mm in diameter. In order to identify proteins in excised gel spots, we used a protein identification service (Protein Works, Daejeon, Korea) with LC-MS/MS analysis. Briefly, proteins were subjected to in-gel digestion. MS/MS of peptide generated by in-gel digestion was performed by nano-ESI on a Q-TOF2 mass spectrometer (AB Sciex instruments, CA94404 USA). To identify the protein, peptide masses from Q-TOF MS were matched with the theoretical peptides of proteins in the NCBI database using MASCOT search program (www.matrixscience.com).

Result

In order to compare the proteomic difference of muscle proteins in Hanwoo and Holstein samples, muscle proteins were separated by 2DE, and the relative protein quantities on the spots measured using 2DE image analysis software (PDQuest, Bio-Rad, USA). As shown in Fig. 1, the total number of protein spots from muscle tissues were slightly higher in Hanwoo (575 $\pm$ 65) than in Holstein (534 $\pm$ 13), but these numbers were not statistically significant, mainly due to the large variation. General protein patterns in 2DE gels were nearly the same in muscle tissues between the two breeds except for several spots. Twelve protein spots were identified as candidate proteins, appearing in one breed's muscle tissue. The proteins on the spots from 1 to 8 were identified in Hanwoo sample (Fig. 2) and 9 to 12 were identified in Holstein one (Fig. 3).

The protein characteristics on the spots were analyzed by LC-MS/MS analysis using NCBI and EST databases using the MASCOT search engine, and the results are presented in Table 1. The proteins on spots 1 to 2 were identified to smooth muscle and non-muscle myosin alkali light chain isomers. Spots 3, 4 and 5 were identified to α B crystallin isomers. The proteins on spots 6, 7, 8 were identified as hemoglobin subunit beta-A, slow myosin heavy chain, and slow skeletal muscle troponin T,

Fig 1. Comparative analyses of protein expressions of the *longissimus dorsi* between Hanwoo and Holstein. Proteins were separated using an IPG 3-10 L in first dimension and 12% SDS gel in the second dimension. The protein was loaded with 800 μ g and the gel was stained with colloidal Coomassie Brilliant Blue G-250 for 48 h. Arrows indicate the spot with different expression levels between two cattle breeds.

Fig. 2. Expression profiles of eight Hanwoo specific protein spots between Hanwoo (Han) and Holstein (Hol) in the *longissimus dorsi*, and comparisons of the protein quantity of the spot. Circles on the gel show the spot density change, with five replicates for each group. The bars represent the average density measured by PDQuest software.

Fig. 2. Continued.

respectively, while spot 9 was identified as ankyrin repeat domain 2. Proteins on spots 10, 11, and 12 were identi-

fied as creatin kinase isomers.

Fig. 3. Expression profiles of four Holstein specific protein spots between Hanwoo (Han) and Holstein (Hol) in the *longissimus dorsi*, and comparisons of the protein quantity of the spot. Circles on the gel show the spot density change, with five replicates for each group. The bars represent the average density measured by PDQuest software.

Discussion

In this study, we found 12 protein spots on the two-dimensional electrophoresis gels stained by CBBG. The proteins on spots 1 to 8 were identified in Hanwoo sample (Fig. 2). By LC-MS/MS analysis, these spots were identified as smooth muscle and non-muscle myosin alkali light chain (MYL) 6B isomers, α B crystallin (α BCRY) isomers, hemoglobin β -A chain (H β bA), slow myosin heavy chain (MyHC), and slow skeletal muscle troponin T (TnT). The MYL 6B isomers on spots 1 and 2

were reported to be found in vascular smooth muscle cells and their functions related to the regulation of blood flow in the skeletal muscle (www.matrixscience.com). Also, Sweeney *et al.* (1987) reported that the concentrations of MYL proteins were proportional to the amount of slow-twitch muscle fiber in skeletal muscle. Therefore, the detection of MYL 6B isomers in Hanwoo beef (but not in Holstein beef) implies that Hanwoo beef contained more slow-twitch muscle fibers than Holstein beef. CRY α B isomers on spots 3, 4, and 5 were identified to be major structural proteins originally identified in the ocu-

Table 1. List of proteins identified by Q-ToF analysis between Hanwoo and Holstein

Spot No.	Protein identified	NCBI gi No.	MW ¹⁾	pI ²⁾	Source	C/Q ³⁾	Peptide sequence
1,2	Smooth muscle and non-muscle myosin alkali light chain 6B	gi 115496556	23,502	5.40	Bos taurus	5/1	IQEPPIDLSK
3,4	Crystalline, alpha B	gi 27805849	20,024	6.76	Bos taurus	14/1	IPADVDPPLAITSSLSSD GVLTVNGPR
5	Alpha B-crystallin	gi 57580	19,945	6.84	Rattus rattus	7/1	APSWIDTGLSEMR
6	Hemoglobin beta-A chain	gi 122539	16,011	6.36	Bos javanicus	8/1	LLGNVLVVVLAR
7	Slow myosin heavy chain	gi 4127664	8,202	9.60	Canis familiaris	18/1	VIQYFAVIAAIGDR
8	Slow skeletal muscle troponin T	gi 339781	32,971	5.85	Homo sapiens	5/1	DLLELQTLIDVHFEQR
9	Ankyrin repeat domain 2	gi 9910130	36,855	5.95	Mus musculus	4/1	TPTDLVQLWQADTR
10	Creatine kinase M	gi 180576	43,247	6.63	Homo sapiens	3/1	LSVEALNSLTGEFK
11	Creatine kinase M-type	gi 125303	43,354	6.77	Canis lupus	4/1	LGSSEVEQVQLVVDGVK
12	Creatine kinase chain	gi 4838363	43,172	6.63	Bos taurus	5/1	GTGGVDTAAVGSVFVDVSNADR

¹⁾MW = molecular weight.

²⁾pI = isoelectric point.

³⁾C/Q; sequence coverage/queries matched.

lar lens (Fujii *et al.*, 2007; Horwitz, 2000; Groenen *et al.*, 1994). It has also been reported to be expressed in skeletal muscle fibers during oxidative stress (Neufer and Benjamin, 1996). Because oxidative stress can be caused by incomplete oxidative phosphorylation in mitochondria (Schluter *et al.*, 2000) and the slow-twitch muscle fiber has a high capacity for oxidative phosphorylation, the detection of α B crystallin (CRY α B) isomers likely mirrors the slow-twitch muscle fibers. Therefore, exclusive expression of CRY α B isomers in Hanwoo *longissimus* muscle indicates that the muscle tissue is composed of more the slow-twitch muscle fibers than Holstein one. MyHC on spot 7 and TnT on spot 8 were reported to be found only in slow-twitch muscle fibers (Laurent-Winter *et al.*, 1991). Therefore, our data also suggested that Hanwoo beef has more slow-twitch muscle fibers relative to Holstein beef, as MyHC and TnT was detected in Hanwoo beef but not in Holstein beef. Overall, the detection of MYL6B isomers, CRY α B isomers, H β bA, MyHC and TnT in Hanwoo beef and not in Holstein beef, implied that Hanwoo beef has more slow-twitch muscle fibers relative to Holstein beef.

The spots 9 to 12 were identified in Holstein sample (Fig. 3). These spots were not detected in Hanwoo beef and were identified as ankyrin repeat domain 2 (Arpp2) and creatin kinase (CK) isomers by LC-MS/MS analysis (Table 1). Arpp2 on spot 9 was reported to be found in striated muscle and in response to muscle stretch (Kemp *et al.*, 2000; Miller *et al.*, 2003). Ohsaki *et al.* (2007) observed that very high levels of Arpp2 proteins were only expressed in Holstein beef, similar to our results. The CK isomers, which catalyzed the reversible transfer of the phosphoryl group from MgATP to creatine, leading

to phosphocreatine and ADP, were known to be related to the most powerful energy production involved in a non-oxygen dependent process responsible for fast-twitch muscle contraction (Darnell *et al.*, 1986). Schmitt and Pette (1985) demonstrated that the concentration of CK isomers were higher in the fast-twitch muscle fibers than the slow-twitch muscle fibers. Therefore, our data, which detected CK isomers in Holstein beef, suggested that Holstein beef has more fast-twitch fibers than Hanwoo beef.

In conclusion, we found twelve protein spots that were differently expressed between Hanwoo sample and Holstein one. These proteins were identified by LC-MS/MS analysis. The result of our study indicates that the Hanwoo beef contained more slow-twitch muscle fibers than Holstein beef and these candidate proteins identified may be possible as an influence on meat quality, although further studies are required to illuminate the differences between two cattle breeds.

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