Meat Science 136 (2018) 35–43

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Differential expression of extracellular matrix and integrin genes in the *longissimus thoracis* between bulls and steers and their association with intramuscular fat contents

Seung Ju Park[a], Hyeok Joong Kang[a], Sangweon Na[a], Seung Hwan Lee[b], Myunggi Baik[a,c,*]

[a] Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, College of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea
[b] Division of Animal and Dairy Science, Chungnam National University, Daejeon 34134, Republic of Korea
[c] Institutes of Green Bio Science Technology, Pyeongchang-gun, Gangwon-do 25354, Republic of Korea

ARTICLE INFO

Keywords:
Castration
Collagen gene
Extracellular matrix
Integrin
Korean cattle
Longissimus thoracis

ABSTRACT

This study was performed to compare expression of genes for extracellular matrix (ECM) components, ECM degrading factors, and integrin subunits in the *longissimus thoracis* (LT) between bulls and steers. Steers had lower ($P < 0.05$) ECM component collagen type 1 α1 and collagen type 3 α1 mRNA levels than did bulls, but they had higher ($P < 0.05$) thrombospondin 1 mRNA and protein levels. Steers had higher ($P < 0.01$) matrix metalloproteinase (MMP) 9 mRNA levels than did bulls. Steers had higher ($P < 0.05$) integrin α5 mRNA levels but lower ($P < 0.05$) integrin β6 mRNA and protein levels; however, expression levels of several other integrin subunits were not different between steers and bulls. MMP9 mRNA levels were positively correlated ($P < 0.05$) with intramuscular fat content in bull group. In conclusion, these results demonstrate that castration has moderate effects on expression of ECM components, ECM degrading factors, and integrin subunit genes in the LT.

1. Introduction

Castration significantly increases the marbling score (MS) and intramuscular fat (IMF) deposition, and thus improves the quality grade (QG) of beef (Bong et al., 2012; Park et al., 2002). Our previous studies showed that activating adipogenesis and lipogenesis is important for increasing IMF deposition following castration (Baik et al., 2017; Baik, Nquyen, Jeong, Piao, & Kang, 2015; Bong et al., 2012; Jeong, Kim, Nguyen, Lee, & Baik, 2013; Jeong, Kwon, Im, Seo, & Baik, 2012).

Fibrogenesis is another mechanism regulating IMF deposition. Du et al. (2013) suggested that muscle cells, adipocytes, and fibroblasts are all derived from the same pool of mesenchymal progenitor cells, which are present in mature muscle, although they are abundant during early developmental stages. The majority of these cells undergo myogenic differentiation, but a significant proportion differentiates into common progenitor cells, committed to either adipocytes and fibroblasts or so-called fibro/adipogenic progenitor cells, which are located in the extracellular matrix (ECM) of muscle fibers (Du et al., 2013). The same authors suggested that enhancing the adipogenic differentiation of these progenitor cells increases IMF, whereas fibrogenic differentiation stimulates the synthesis of ECM components or connective tissue in muscle. Japanese Wagyu muscle has higher IMF and collagen contents compared with those of Angus muscle, and this is accompanied by increased adipogenesis and fibrogenesis in Wagyu muscle compared with Angus muscle (Duarte et al., 2013). Castration may affect the regulation of fibrogenic gene expression. However, little is known about the molecular changes associated with fibrogenesis in the *longissimus dorsi* (LM) following castration of bulls.

Marbling adipocytes and connective tissue, such as collagen and fibronectin, are present in the ECM of the LM (Duarte et al., 2013). The amount of IMF deposition and the collagen contents are higher in Wagyu muscle than in Angus muscle (Duarte et al., 2013). Thrombospondin 1 (THBS1) is a matricellular ECM protein that interacts with other ECM proteins, such as collagens (Mumby, Raugi, & Bornstein, 1984). Thrombospondin 1 is also an adipokine that is highly expressed in obese human subjects (Varma et al., 2008). No study has investigated THBS1 during adipogenesis of cattle. Changes in the expression of genes for ECM components may affect the degree of marbling following castration.

Matrix metalloproteinase (MMP) family members are essential

* Corresponding author at: Department of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 08826, Republic of Korea.
E-mail address: mgbaik@snu.ac.kr (M. Baik).

http://dx.doi.org/10.1016/j.meatsci.2017.10.008
Received 20 June 2017; Received in revised form 6 October 2017; Accepted 11 October 2017
Available online 14 October 2017
0309-1740/

enzymes to degrade the ECM, and they are considered to play important roles in adipogenesis and fibrogenesis in rodent studies (Bouloumié, Sengenès, Portolan, Galitzky, & Lafontan, 2001; Chavey et al., 2003; Sternlicht & Werb, 2001). Among MMP family members, MMP9 is implicated as an important regulator of fat deposition (Bouloumié et al., 2001; Bourlier et al., 2005). Matrix metalloproteinase 13 degrades collagen (López-Rivera et al., 2005). In their review, Christensen and Purslow (2016) suggested important roles for MMP in the regulation of myogenesis, fibrogenesis, and adipogenesis, thereby possibly affecting meat quality in domestic animals. Matrix metalloproteinases are downregulated by tissue inhibitor of metalloproteinase (TIMP) family members (Balcerzak, Querengesser, Dixon, & Baracos, 2001; Parsons, Watson, Brown, Collins, & Steele, 1997), and TIMP is implicated in adipose tissue remodeling (Fenech, Gavrilovic, & Turner, 2015). Matrix metalloproteinases and TIMP may have functions in regulating IMF deposition and fibrogenesis in cattle.

The integrin family of adhesion receptors is comprised of cell surface proteins that mediate adhesion to the ECM and cell-cell interactions (Hynes, 2002). Integrins are heterodimeric transmembrane proteins comprised of α and β subunits (Legate, Wickström, & Fässler, 2009). Extracellular matrix components, including fibronectin and THBS1, exert their biological effects through specific cell surface receptors, such as integrins (Dean, Birkenmeier, Rosen, & Weintraub, 1991; Lawler, 2000). One study reported that integrins regulate the proliferation and differentiation of human adipose-derived stem cells (Morandi et al., 2016). Integrin signaling through ECM components may help control adipogenesis and fibrogenesis in the LM of cattle.

However, limited information is available on the role of ECM-associated factors in IMF deposition in the LM. Furthermore, little is known about the molecular changes associated with fibrogenesis, ECM components, ECM degrading factors (MMP and TIMP), and integrins in the LM following castration of bulls. We hypothesized that in addition to adipogenesis, castration affects gene expression of ECM components and its associated genes, contributing to IMF deposition, MS, and QG of beef. This study was performed to understand the transcriptional changes in fibrogenic factor, ECM component, ECM degrading factor, and integrin receptor genes in the LM following castration of bulls. The relationships between gene expression levels and IMF contents were also investigated.

2. Materials and methods

All experimental procedures involving animals were approved by the Chonnam National University Institutional Animal Use and Care Committee (CNUIAUCC: CNU IACUC-YB-2010-9), Republic of Korea. The experiments were conducted in accordance with the Animal Experimental Guidelines of CNUIAUCC.

2.1. Animals and tissue samples

In this study, we used *longissimus thoracis* (LT) samples to determine the expression levels of genes in all tissues in 10 bulls and 10 Korean cattle steers from a previous study (Bong et al., 2012). The feeding method was described previously, and the carcass characteristics have been reported by our laboratory (Bong et al., 2012). Of the 20 steers, we used the 10 with the highest marbling scores (6–9) on a scale of 1–9, with 1 being the lowest and 9 the highest marbling score, to examine differential gene expression by marbling score. We used the same animals as Jeong et al. (2013) except two steers because of a lack of tissue samples. The mean marbling score (1.1 vs. 7.2 in bulls and steers) and QG were significantly higher ($P < 0.001$) in steers compared to bulls. The mean slaughter age and mean carcass weight of bulls and steers were 20 and 28 months, and 347 kg and 414 kg, respectively.

Intramuscular fat content was measured following the procedure of Folch, Lees, and Sloane Stanley (1957). Briefly, LT tissues were ground to a fine powder and homogenized in a 2:1 chloroform-methanol mixture (vol/vol). The fat-containing solvents were evaporated, and the fat content was measured. Nine steer samples were analyzed for IMF contents due to a lack of tissue sample of one steer. Steer LT had 4.0-fold greater ($P < 0.001$) IMF content (16.6 ± 1.83%) compared with bull LT (4.2 ± 0.53%).

We separated the muscle and IMF portions from the LM of the steers to evaluate the effect of IMF content on gene expression in the LT, as described previously (Bong et al., 2012).

2.2. RNA extraction and quantitative real-time polymerase chain reaction (qPCR)

Total RNA was isolated from tissues using TRIzol Reagent (Molecular Research Center, Cincinnati, OH, USA), according to the manufacturer's instructions. RNA concentration was measured using a NanoPhotometer (Implen GmbH, Schatzbogen, München, Germany). The integrity of total RNA was initially verified through ethidium bromide staining of the 28S and 18S agarose gel electrophoresis bands. RNA integrity was also verified using a Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA): an RNA integrity number (RIN) ≥ 8.0 was considered acceptable. RNA was stored at −70 °C until analysis.

Total RNA was reverse-transcribed into cDNA using the iScript cDNA synthesis kit (Bio-Rad Laboratories Inc., Hercules, CA, USA), according to the manufacturer's instructions. Reverse transcription was conducted in a 10-μL total reaction volume that contained 2 μg RNA template, 2 μL of 5 × iScript Reaction Mix, 0.5 μL of iScript reverse transcriptase, and 2.5 μL of nuclease-free water. The thermal parameters were: 25 °C for 5 min, 42 °C for 30 min, and 85 °C for 5 min.

The qPCR was performed as reported previously (Bong et al., 2012; Jeong et al., 2013) using QuantiTect SYBR Green RT-PCR Master Mix (Qiagen, Valencia, CA, USA). We followed the “Minimum Information for Publication of Quantitative Real-Time PCR Experiments” (MIQE) guidelines for qPCR as much as possible (Bustin et al., 2009). All qPCR analyses were conducted in a 25-μL total reaction volume that contained 20 ng cDNA, 12.5 μL SYBR Green RT-PCR Master Mix, and 1.25 μL of 10 μM primers. The thermal cycling parameters were: 95 °C for 15 min, followed by 40 cycles at 94 °C for 15 s, 55 °C for 30 s, and 72 °C for 30 s. Primer information is presented in Supplementary Table 1. We used two different exons for forward and reverse primers to prevent amplification of the DNA template. We indicated the melting temperatures (Tm) of all primers to Supplementary Table 1. The Tms of all of the primers were 57.0–61.5 °C. Thus, an annealing temperature of 55 °C was used for amplification of all of the genes, resulting in a single major peak in all cases. We tested primer amplification efficiency for all genes according to Bustin et al., 2009. Briefly, cDNA was serially diluted, qPCR was performed as described above, and a calibration curve was generated as follows: the logarithm of the cDNA template concentration was plotted on the *x*-axis and the quantitation cycle (Cq) was plotted on the *y*-axis. PCR efficiency was determined from the slope of the linear portion of the log calibration curve; PCR efficiency = $10^{-1/\text{slope}} - 1$. Primer efficiency for all genes was 90–110%, which is acceptable for qPCR (Supplementary Table 1). We used a similar primer efficiency test method in a previous study (Ahn et al., 2014). The ΔΔCT method was used to determine the relative fold change in gene expression (Livak & Schmittgen, 2001). In this study, we evaluated whether β-actin, ribosomal protein lateral stalk subunit P0 (RPLP0), and 18s RNA gene are suitable reference genes. β-actin expression was generally uniform in the LT between bulls and steers, whereas RPLP0 expression was uniform in both the muscle and IMF portions of the LT. Therefore, β-actin was used as the reference gene in the LT, and RPLP0 was used in comparisons of the IMF and muscle. We used β-actin as a reference gene in the LT in two previous studies of bulls and steers (Bong et al., 2012; Jeong et al., 2013). Ribosomal protein lateral stalk subunit P0 is reportedly one of the most stable reference genes in bovine adipose tissue, muscle, and mammary glands (Bonnet, Bernard,