

Effects of early high nutrition related to metabolic imprinting events on comprehensive DNA methylation of longissimus muscle in grass-fed Wagyu

Daichi Nishino ¹, Sithyphone Khounsaknalath ¹, Kunihiro Saito ², Akira Saito ³, Tsuyoshi Abe ², Shinobu Yasuo ¹, Takafumi Gotoh ^{1,4}

¹ Kyushu University, ² National Livestock Breeding Center, ³ Zenrakuren, ⁴ Kagoshima University, Japan

Objectives: Wagyu (Japanese black cattle) has a high potential to accumulate intramuscular fat (IF). Japanese farmers require a large amount of grain to maximize their ability of marbling. Not only in Japan but also various countries such as the United States and Australia, the feedlot system is a standard raising. On the other hand, grassing is reevaluated as a sustainable system maintaining ecosystem and landscape. Compared with grain-fed beef production, the efficiency of grass-fattening was inferior. However, it has been reported that early high nutrition related to metabolic programming or epigenetics positively affects the growth, carcass characteristics, and meat quality of grass-fed Wagyu. Discovering functional genes and corresponding regulatory pathways related to these phenotypes based on epigenetics is essential to the development of the beef production system. It has been reported that epigenetic modification, especially DNA methylation is related to human diseases and long-term phenotypes. However, the epigenetic mechanisms related to beef quality and quantity have not been elucidated. This study aimed to characterize the profiles of DNA methylation of longissimus muscle (LM) from Wagyu which showed different terminal phenotypes led by manipulating only early high nutrition.

Materials and Methods: Wagyu steers were randomly assigned to 2 dietary groups: a roughage group (RG, n = 11) that provided milk replacer (crude protein 26.0%, crude fat 25.5%; maximum intake 0.6 kg/d) until 3 months of age and then roughage (orchard grass hay) ad libitum from 4 to 10 months of age; and an early high nutrition group (EHN, n = 12) that provided milk replacer (maximum intake of 1.8 kg/d) until 3 months of age and then a high-concentrate diet from 4 to 10 months of age. After 10 months of age, all steers were raised under the same program, which provided roughage ad libitum until 31 months of age and then slaughtered. The body weight (BW) and the IF content were measured. The methylation status of LM was analyzed by whole-genome bisulfite sequencing (WGBS).

Results and Discussion: BW and the IF content were greater in EHN than in RG (both, $p < 0.05$; Khounsaknalath et al. 2021.). Compared with RG, 673 and 764 genes were hypomethylated and hypermethylated in EHN, respectively ($|\text{difference of methylation rate}| > 25\%$, $q \text{ value} < 0.01$). A total of 10721 differentially methylated regions (DMRs) were identified between RG and EHN ($|\text{difference of methylation rate}| > 25\%$, $q \text{ value} < 0.01$). In particular, 5064 DMRs were hypomethylated including 161 in the 1000 to 5000bp upstream region of the transcription start site (1 to 5kb), 154 in the promoter, 181 in the 5'UTR, 37 in exons, 948 in introns, 238 in the boundary between intron and exon, 68 in the 3' UTR and 3277 in the intergenic region. 5657 DMRs were hypermethylated including 197 in 1 to 5kb, 163 in the promoter, 198 in the 5' untranslated region (UTR), 58 in exons, 1091 in introns, 275 in the boundary between intron and exon, 104 in the 3' UTR and 3571 in the intergenic region. The differences in the methylation level were observed in functional genes, such as CD44, Hepatocyte growth factor (HGF), PRDM family, FOX family, and Solute carrier (SLC) family. Especially CD44, which is hypomethylated in 1 to 5kb, is a key factor that stimulates cell proliferation and migration. In addition, PRDM protein, which is coded by the PRDM family, has a SET domain that belongs to the DNA methyltransferase family. These genes might have potential application in producing high quality and a large quantity of beef. Our investigations reveal that early high nutrition epigenetically affects the DNA methylation status of LM from Wagyu. These results provide not only novel epigenetic information related to grass-fed Wagyu but also valuable insight into meat science. To further explore the molecular mechanisms related to phenotypic differences in Wagyu, analyses of the gene expression, protein, and metabolome are required.

Key words: DNA methylation, Wagyu, metabolic imprinting, Whole-genome bisulfite sequencing, Epigenetics