Methylation modification of genomic DNA followed to induction of differentiation in bovine intramuscular preadipocyte cells from Japanese Black cattle.

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Abstract

Meat quality of Japanese Black cattle (JB) are known as a excel character in Wagyu, and has become very popular worldwide. However the main mechanism of developing their characteristic features is not well understood although many researchers have them reported SNPs and QTLs which may be concerning with them. DNA methylation to C in CG rich of the upstream region of start codon controls the expression of many genes on a genome wide level with reflecting to environmental effects. This study aims to clarify regions modified by methylation or demethylation in genomic DNA followed to induction of differentiation in bovine intramuscular preadipocyte cells from Japanese Black cattle. Bovine intramuscular preadipocyte cells, BIP established from Japanese Black cattle was cultured and induced the differentiation by following to a methods by Aso et.al, 1995. Genomic DNA from BIP was extracted by using an extraction kit and one for lipid, Takara Bio. And Methylation analysis was performed by using Infinium Methylation EPIC Bead Chip in Takara Bio. 860,000 of regions in genomic DNA of BIP cells were detected. 183,815 regions of 860,000 were estimated as CG rich region. DNA methylation in 120 of regions changed significantly according to the induction of differentiation. Genes controlled by the promoters which had modification changed in CpG might relate to lipid accumulation or differentiation.

Object

Today, we know that DNA methylation to CG rich regions in the upstream region of a start codon, ATG, controls the expression of many genes on a genome wide level, and a part of methylation patterns (DMP) is specific by each tissue and differentiation stage. It is, therefore, important to include DMP should be included as a significant effect to evaluate accurately bovine's performances accurately. In the previous research, we reported relationships among DMP at the slaughter after fattening and economic carcass traits of JB siblings of two sires. Followed to these results, this study aims to clarify regions modified by methylation or demethylation in genomic DNA followed to induction of differentiation in bovine intramuscular preadipocyte cells from Japanese Black cattle. In this study, although apparent relationships between the known QTL and this gene could not be found, this information might be an effective marker for monitoring meat quality in JB.

Material and Method

BIP and induction to adipocyte. Bovine intramuscular preadipocyte cells (BIP) established from Japanese Black cattle was cultured and induced the differentiation to adipocyte by following to a methods by Aso et.al, 1995. Genomic DNA from BIP was extracted by using an extraction kit and one for lipid, Takara Bio. And Methylation analysis was performed by using Infinium Methylation EPIC Bead Chip in Takara Bio. 860,000 of regions in genomic DNA of BIP cells were detected

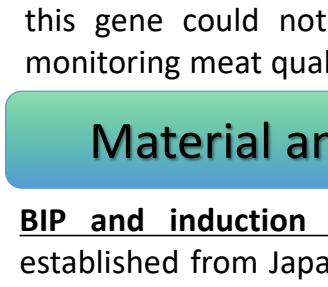
mRNA expression for related to lipid storage and cell cycle.

To evaluate gene expressions for related to lipid storage and cell cycle, PPARg, ap2, SREPB1, ASS1, SCD, CHFR mRNA production were evaluated by the realtime PCR method and β-actin mRNA production as internal criteria.

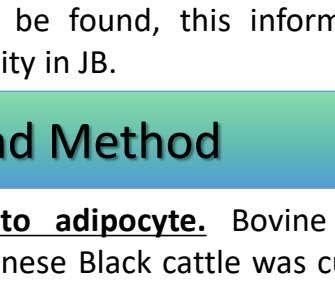
Data calculation and statistical analyses. To evaluate all data, a specific application published GenomeStudio by Illumina was used, and general statistic analysis are performed by using SAS program, version 9.1 in accordance with its operational manual.

Result and Discussion

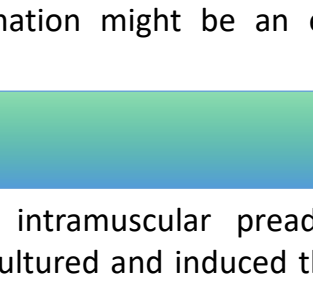
BIP and induction to adipocyte.



Day 0 of induction
BIP cultured

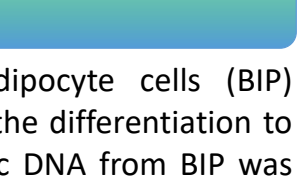


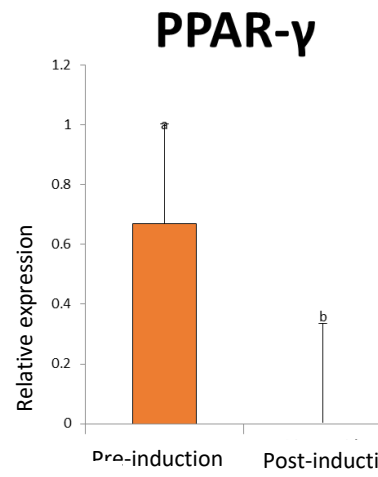
Day 6 of induction
BIP cultured

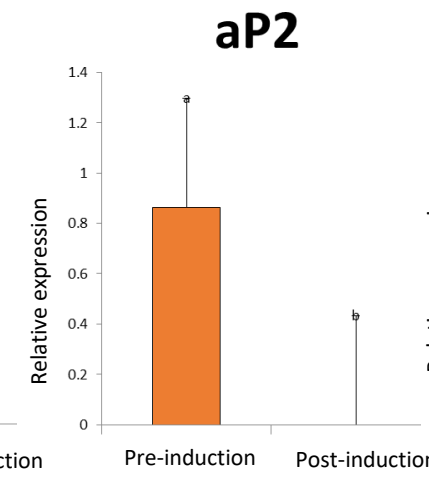


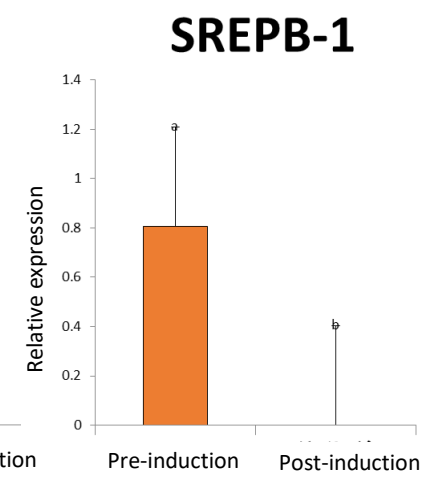
Day 7 from stimulus
(differentiation; adipocyte)

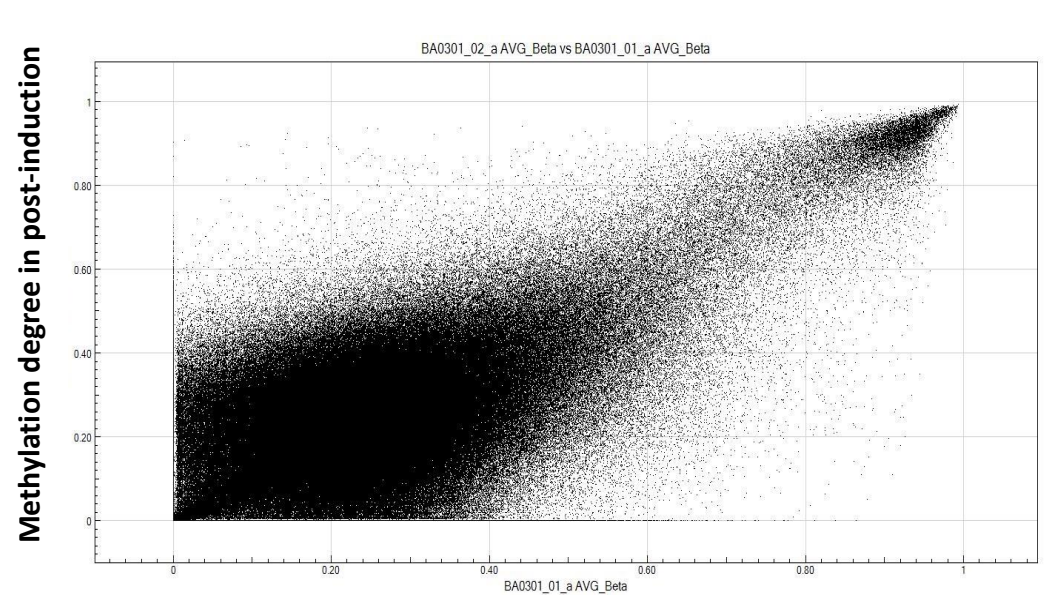
Extraction of Genomic DNA



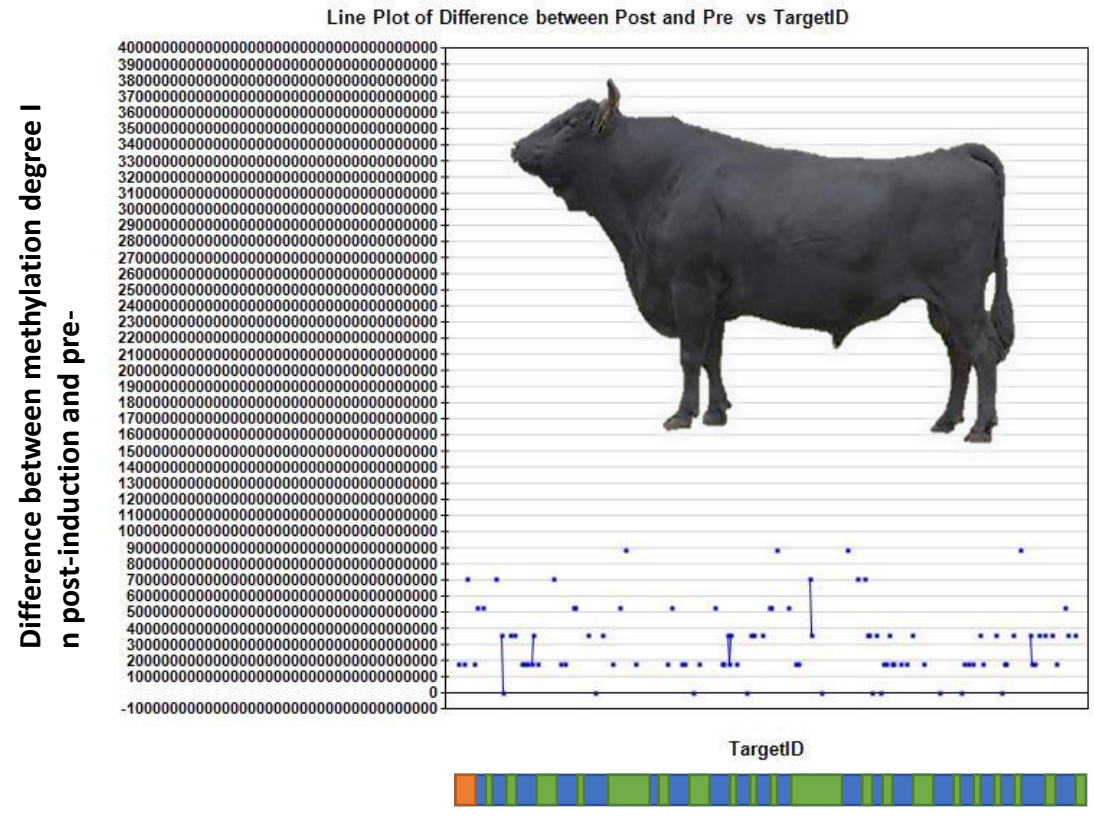






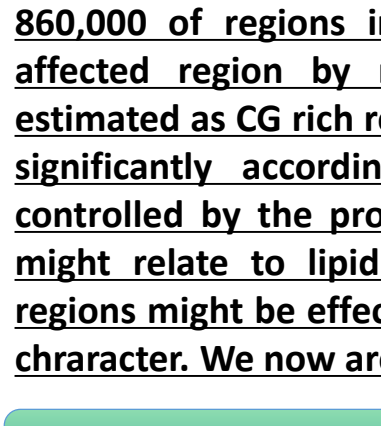


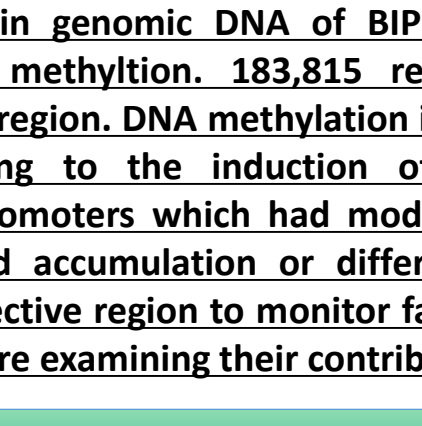
Methylation degree in pre-induction

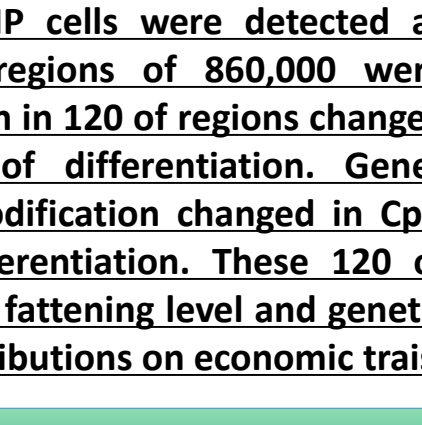


865,968 of methylation regions known in human

860,000 of regions in genomic DNA of BIP cells were detected as affected region by methyltion. 183,815 regions of 860,000 were estimated as CG rich region. DNA methylation in 120 of regions changed significantly according to the induction of differentiation. Genes controlled by the promoters which had modification changed in CpG might relate to lipid accumulation or differentiation. These 120 of regions might be effective region to monitor fattening level and genetic chraracter. We now are examining their contributions on economic trais.







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Acknowledgement

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