EPA activates PPARδ and AMPK pathways in L6 myotubes

Yusuke Komiya [1], Wataru Mizunoya [2], Keizo Arihara [1]

[1] *School of Veterinary Medicine, Kitasato University,* [2] *School of Veterinary Medicine, Azabu University, Japan*

Objectives: Skeletal muscle fibers are classified into type 1 (slow-twitch) and type 2 (fast-twitch) fibers. The difference of muscle fi- ber type composition affects several parameters related meat quality. We previously reported a significant positive correlation be- tween the composition of type 1 fibers and umami taste of beef samples in taste sensor analysis. The final goal of our study is to produce livestocks whose muscle is rich in type 1 fibers by feeding control, and it contributes to improve meat quality. Our previous study has demonstrated that dietary fish oil intake induced the fast-to-slow alterations of muscle fiber type composition in rats. Here, we focused on the eicosapentaenoic acid (EPA) contained in fish oil. The objective of this study is to examine the effects of EPA on muscle cells by comprehensive analysis with metabolomic and transcriptomic approaches in rat myotubes. Furthremore, we compared the cellular responses between EPA and PPARδ activation which is known to induce slow-twitch fiber formation.

Materials and Methods: Culture: Rat myoblast cells (L6) were cultured in growth medium [DMEM supplemented with 10% FBS, 1% antibiotic-antimycotic mixed stock solution, and 0.5% gentamicin] to reach 80-90% confluency. After reaching confluence, the cells were cultured in differentiation medium [DMEM supplemented with 2% horse serum (HS; Invitrogen), 1% antibiotic-antimycotic mixed stock solution, and 0.5% gentamicin] for 10 days to form myotubes. Differentiated myotubes were treated with 30 μM eicosapentaenoic acid (EPA group) or 100 nM GW501516 (a PPARδ selective agonist, GW group) for 12h or 96h in RNAseq and metabolome analysis respectively. RNAseq: Total RNA was extracted from L6 myotubes using the NucleoSpin RNA according to the manufacturer's protocol. RNA-seq libraries were prepared by Truseq stranded mRNA Library (Illumina) and obtained cDNA were subjected to 100-bp pairedend sequencing on Illumina NovaSeq6000 platform. The reads were aligned to rat reference genome obtained from Ensembl database and mapped reads were counted and gene expression level were analyzed using HISAT2, featureCounts and edgeR. Principal component analysis (PCA) and Hierarchical cluster analysis (HCA) were performed by R pro- grams. The gene ontology (GO) and pathway analysis were performed using DAVID software. Metabolomics: Metabolome analy- sis was performed at Human Metabolome Technologies, Inc (HMT). Cellular metabolites were extracted using methanol contain- ing HMT internal standard solution accorind to the manufacturer's instruction. Analysis was conducted according to HMT's C- SCOPE package, using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) for cation analysis and CE-tandem mass spectrometry (CE-MS/MS) for anion analysis. PCA and HCA were performed by R programs and detected metabolites were plotted on metabolic pathway maps using VANTED software.

Results and Discussion: RNAseq: The PCA demonstrated clear clustering in three control, EPA and GW groups. The separation was profound between EPA and other groups (PC1), suggesting that EPA treatment induced a significant change in the transcript pro- files of the muscle cells independent to PPARδ activation. The HCA of the transcriptomes in myotubes showed a remarkably different pattern in three groups. The results of the HCA classification were very similar to those of the PCA analysis. In EPA group, there were 62 differentially expressed genes compared to controls, including 42 upregulated (Log2FC > 1.0) and 20 downregulated (Log2FC < -1.0) genes. Approximately 50% of the regulated genes were common between EPA and GW501516, demonstrating that EPA treatment partially mimics PPARδ agonism in transcript level. The gene ontology (GO) and pathway analysis of regulated genes revealed significant enrichment in cellular response to insulin stimulus, PPAR signaling pathway, AMPK signaling pathway, mitochondrion, and cellular response to fatty acids (FA) in EPA group, and in mitochondrion, PPAR signaling pathway, thermogen- esis, cellular response to FA, and regulation of FA oxidation in GW group. EPA treatment is more intensely engaged in the cellular response to insulin stimulus and AMPK signaling pathway than GW group, suggesting that these pathways were induced by EPA independently from PPARδ activation. The upregulated pathways related to mitochondria and FA metabolism were common be- tween EPA and GW group. Metabolomics: The 116 peaks (52 cations and 64 anions) were detected by the anion and cation modes of CE-QqQ/TOFMS. The three control, EPA and GW groups were also clearly distinguishable in PCA and HCA, the classifications were very similar to those of the transcriptome analysis. Focusing on the individual metabolites, the levels of citric acid and cis- Aconitic acid decreased, and succinic acid, relating TCA cycle, increased in EPA group compared to control group. We next focused on glycolysis and lipid metabolism, the levels of G6P and F6P were decreased and the levels of 3-PG, 2-PG, PEP, and lactic acid were increased in EPA treatment compared to control group. These alterations of metabolites in glycolysis were not accordance with GW group. Taken together, our comprehensive transcriptome and metabolome analyses strongly suggested that EPA regulated muscle metabolic characteristics through AMPK and PPARδ activation, and this combined effect might enhance new type 1 fiber formation.

Key words: EPA, Metabolome, Transcriptome, PPARδ, Myotube