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IDENTIFICATION OF PROTEIN LACTYLATION IN POSTMORTEM PORK

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I. INTRODUCTION

Lactate-derived lactylation, a newly found protein post-translational modification in 2019, occurs on lysine residue of protein. It regulates the activity, stability, and interaction of proteins playing a critical role in cellular biochemical processes [1-2]. Our previous study for the first time has shown that the degree of protein lactylation in pale, soft, and exudative (PSE) pork was significantly higher relative to normal pork, implying that protein lactylation might be potentially involved in the development of meat quality [3]. This work aimed to further explore the spatial localization of lactyltransferase, including E1A binding protein p300 and cAMP response element binding protein (p300/CBP), and lactylated proteins in postmortem myocytes as well as to identify their involvement with critical metabolic processes in postmortem pork.

II. MATERIALS AND METHODS

2.1 Sample collection

Castrated Duroc × Landrace × Yorkshire crossbred pigs (6 months old, 120 ± 10 kg) were slaughtered at a commercial slaughter plant (Sushi Meat Products Co., Ltd., Huai'an, China) following the Chinese national slaughtering standards (GB/T 17236–2019). At 1 h postmortem, the *longissimus thoracis* muscles ($6.2 \leqslant pH \leqslant 6.3$) at the 13th to 14th rib were collected and stored at -80 °C for further analysis (n = 3).

2.2 Localization of p300/CBP and lactylated proteins

Localization of p300/CBP and lactylated proteins in postmortem myocytes was detected by immunofluorescence as described by Ma et al [3].

2.3 Identification of lactylated proteins

Firstly, tissue samples were ground into powder using liquid nitrogen to prepare tryptic digested peptides. Then, the lactylated peptides were enriched and purified using L-lactyl lysine antibody and biomaterials (PTM-1401, PTM BIO, China). Finally, LC-MS/MS were performed on a Orbitrap Astral mass spectrometer coupled with Vanquish Neo UHPLC system (Thermo Fisher Scientific, USA) to qualitatively and quantitatively identify lactated proteins.

2.4 Statistical analysis

Mass spectrometry data were searched against the UniProtKB. For peptide and protein identification, false discovery rate (FDR) was set to 1%. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were carried out with the Fisher's exact test, while enriched pathways were statistically significant at the $p < 0.05$ level.

III. RESULTS AND DISCUSSION

As Figure 1A shows, the p300 (shown in red), CBP (shown in green), and lactylated protein signals (shown in yellow) overlaid each other, producing an orange color composite which was primarily localized around the nucleus, sarcolemma, and cytoplasm. More intuitively, the fluorescence signal patterns of p300/CBP and lactylated proteins were almost identical, with lactylated proteins showing no signal in the absence of p300/CBP while displaying a strong signal with the high presence of p300/CBP. This suggests that p300/CBP was predominantly lactyltransferase for protein lactylation in postmortem muscle.

Additionally, according to lactylproteome profiling, 4085 lactylated lysine-sites from 218 proteins were detected and quantified in postmortem pork. The primary cellular components of lactylated proteins were associated with cytoplasm, mitochondrion, supramolecular fiber, contractile muscle fiber, and actin cytoskeleton (Figure 1B). It indicates that protein lactylation might be implicated in a broad range of cellular structures and actively participate in postmortem muscle contraction. The enrichment of pathways participating in postmortem muscle metabolism included carbon metabolism, glycolysis/gluconeogenesis, citrate cycle, oxidative phosphorylation, and biosynthesis of amino acid. These suggest that postmortem energy metabolism was a highly responsive pathway for lactylation modification. All in all, bioinformatic analysis of lactylated proteins showed that energy metabolism and muscle

contraction were critical postmortem biochemical pathways regulated by protein lactylation, which might together serve as crucial roles in pork quality development.

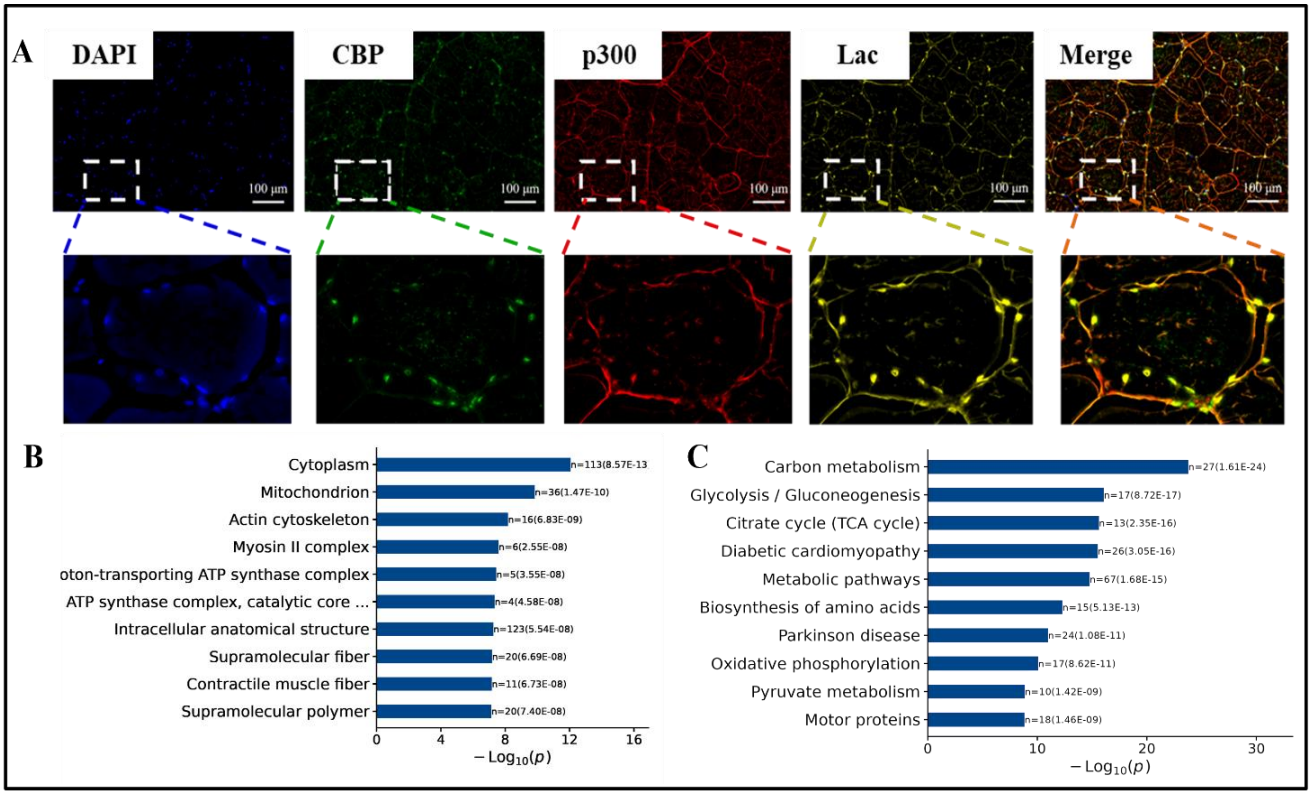


Figure 1. Localization of p300/CBP and lactylated proteins (A) as well as lactylproteome profiling (B and C) of postmortem pork

Notes: DAPI, 4',6-diamidino-2-phenylindole; CBP, cAMP response element binding protein; p300, E1A binding protein p300; Lac, Lactylated proteins. Figure 1B and Figure 1C show functional enrichment profiles of cellular component and biological process terms of lactylated proteins in postmortem pork, respectively.

IV. CONCLUSION

Overall, our work for the first time provided direct evidence for the involvement of protein lactylation in postmortem pork and revealed that energy metabolism and muscle contraction were critical postmortem biochemical pathways regulated by protein lactylation.

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