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DYNAMIC SHIFTS IN METABOLIC DEMAND DURING MYOGENIC PROGRESSION IN PORCINE SKELETAL MUSCLE STEM CELLS

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I. INTRODUCTION

The metabolic processes that govern muscle stem cell proliferation and differentiation play a crucial role in determining muscle growth, fiber type composition, and ultimately meat characteristics [1]. While conventional meat production relies on these natural biological processes occurring *in vivo*, understanding the underlying metabolic mechanisms remains essential for both traditional and emerging meat production systems.

Muscle development follows a coordinated series of events where stem cells proliferate, differentiate, and eventually form functional myofibers. During this progression, significant metabolic reprogramming occurs to meet the changing energy demands and biosynthetic requirements of the cells [2]. These metabolic shifts directly influence muscle fiber formation, which has implications for meat structure and quality attributes such as tenderness and texture.

Metabolomics provides a powerful approach for investigating the complex cellular metabolic processes that underlie muscle development [3]. Therefore, this study aims to explore the dynamic changes in metabolic demands during the proliferation and differentiation stages of porcine skeletal muscle stem cells (PSCs).

II. MATERIALS AND METHODS

PSCs were isolated from *biceps femoris* muscle of 14-d-old female Berkshire piglets according to Kim et al. [4]. The cells were cultured on laminin-coated plates and sampled at proliferation days 1 and 3 (P1, P3) and differentiation days 1 and 2 (D1, D2). Cell characterization was performed using qPCR for myogenic marker expression. Metabolomic analysis utilized UHPLC-Q-Exactive Orbitrap MS for intracellular metabolites, while glycolytic metabolites were quantified with commercial assay kits. All experiments were conducted at least in triplicate (n = 3) with independent trials using PSCs isolated from the same animal. Data were analyzed using one-way ANOVA.

III. RESULTS AND DISCUSSION

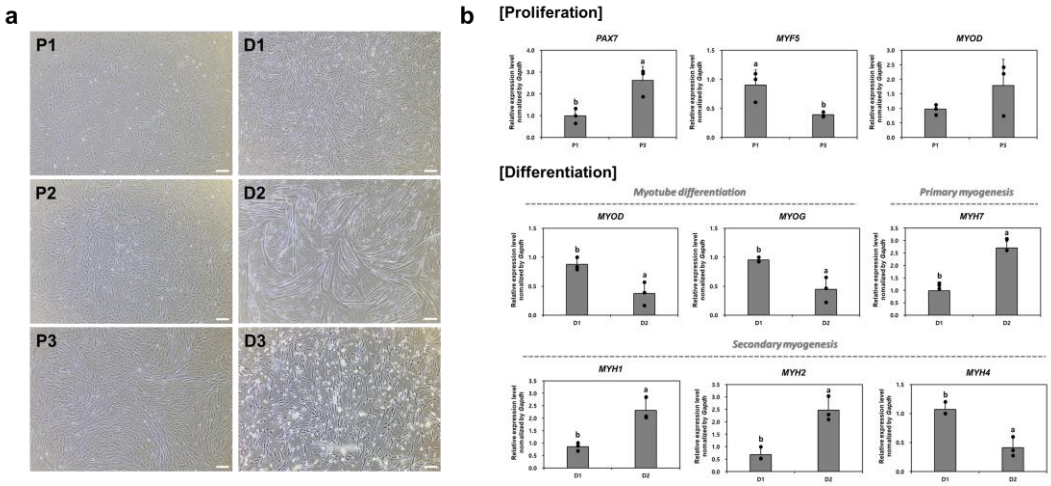


Figure 1. Growth characteristics and myogenic marker expression during proliferation and differentiation of PSCs.

As proliferation progressed, PSCs significantly increased in number, showing typical morphological expansion on the culture plate (Figure 1a). Myotubes first appeared on D2 but detached by D3 due to insufficient structural support in the 2D culture environment, limiting our observations to the early differentiation stages.

Gene expression analysis revealed stage-specific regulation of myogenic markers (Figure 1b). During proliferation, the expression of *PAX7* increased, while *MYF5* decreased significantly in P3 compared to P1, likely due to a transient increase in PAX7 expression during late proliferation stages as PAX7+ cells prepare for myotube. During differentiation, the expression of genes associated with specific muscle fiber types showed significant changes, with

MYH1 (type IIx), *MYH2* (type IIa), and *MYH7* (type I) increasing at D2 compared to D1, whereas *MYH4* (type IIb), *MYOD*, and *MYOG* decreased by D1 (P<0.05).

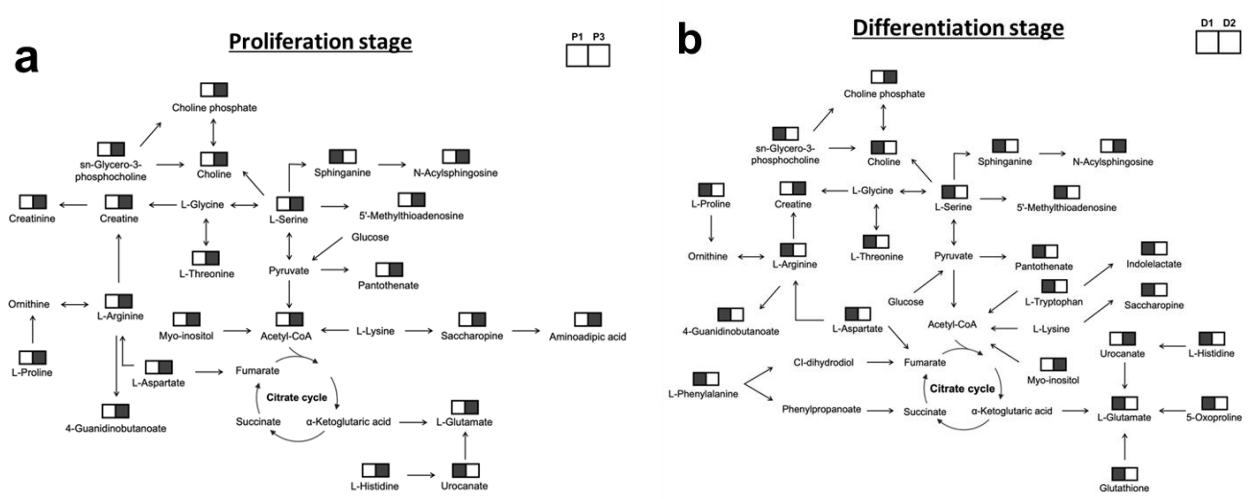
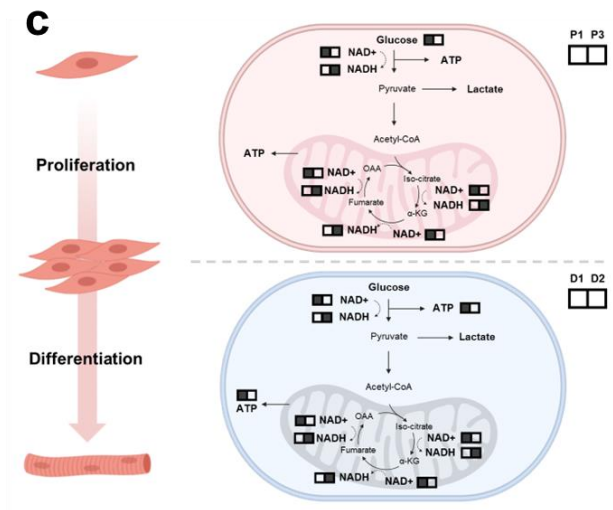


Figure 2. Changes in intracellular metabolomic profiles of PSCs during myogenic progression. A black-filled box over individual metabolites indicates that they are abundant at the corresponding growth stage.



Metabolomic analysis identified 94 intracellular metabolites, primarily consisting of amino acids and peptides. Principal component analysis clearly separated the proliferation stages from differentiation stages, indicating distinct metabolomic profiles during myogenesis (data not shown). Pathway analysis revealed amino acid metabolism as crucial throughout myogenic development, with specific pathways activated at different stages. During proliferation, alanine, aspartate, and glutamate metabolism was predominantly involved, while during differentiation, D-glutamine and D-glutamate metabolism was most significant (data not shown). These amino acid pathways were closely linked to energy metabolism processes such as the tricarboxylic acid cycle and glycolysis (Figures 2a and 2b). Glycolytic metabolites showed a metabolic shift from glycolysis during proliferation to mitochondrial oxidative phosphorylation during differentiation (Figure 2c). This shift aligns with the upregulation of oxidative fiber-type genes at D2, suggesting coordinated regulation between metabolic demands and myogenic differentiation.

IV. CONCLUSION

Distinctive metabolic reprogramming was observed during myogenic progression of PSCs, with a marked shift from glycolysis-dependent energy production during proliferation to mitochondrial oxidative phosphorylation during differentiation. Understanding these stage-specific metabolic requirements provides valuable insights for developing targeted nutritional strategies to enhance muscle development and meat quality in livestock production systems.

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