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Protein Co-Expression Network: A new insight to evaluate molecular mechanisms involved with intramuscular fat deposition and composition (#66)

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Introduction

The nutritional quality and organoleptic properties of meat are mainly affected by the amount and composition of fat in muscle [1]. Beef fat is a source of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids, which are functional ingredients to prevent cardiovascular disease [2]. Several studies have shown that the animal's diet and genetic background significantly affect the meat fatty acid profile [3, 4]. However, the physiological and molecular processes by which these fatty acids are differentially stored in ruminant muscle remains incompletely understood. Proteins are the functional molecules within a tissue and the principal component of skeletal muscle [5], playing a pivotal role for many meat quality attributes. Therefore, to obtain new insights into the biological mechanisms involved in intramuscular fat deposition and composition in Nelore skeletal muscle, a co-expression network with protein quantification data was constructed.

Methods

The population used in this study was 106 Nelore steers raised on pasture and finished in feedlots under identical nutritional and handling conditions. The animals were slaughtered at an average live weight of 452 ± 50 kg and 24 ± 1 month of age. Samples were taken from the *Longissimus* muscle (12th-13th ribs) from each animal, at slaughter, for proteomic analysis, and at 24 hours after slaughter for the intramuscular fat (IMF) and fatty acid content analysis [6,7].

Proteins extraction from muscle were performed as described in [8]. The tryptic peptides were analyzed using a nanoACQUITY UPLC 2D Technology system coupled to Synapt G2-S High Definition Mass Spectrometer (HDMS) (Waters, Manchester, UK). A total of 500 ng of protein digests was loaded on a column for each of the 3 fractions (500 ng/fraction/load). MS data were acquired with Waters MassLynx v.4.1 software and processed using ProteinLynx GlobalSERVER v2.5 (Waters, Manchester, UK). Protein identifications were obtained by searching against a Nelore transcriptome database built from RNA-sequencing data from LD muscle [8]. A maximum false discovery rate (FDR) was set to 4%. Label-free protein quantification values were generated based on the label-free Hi3 method. Proteins were selected based on the quantification in at least 80% of the samples. Merged spectra were normalized to the sum of all intensities.

Co-expression approach was applied using the WGCNA R package version 1.63 [9]. Initially, Pearson's correlations were calculated between each protein pair, followed by transformation to a signed adjacency matrix (AM) by using the soft thresholding power = 10 (R^2 = 0.74), to which co-expression similarity is raised. After, the AM was transformed into a Topological Overlap Matrix (TOM), for which the corresponding dissimilarity (1-TOM) was determined. The modules of highly co-expressed proteins were constructed using a hierarchical clustering tree. The modules were merged based on the dissimilarity of their eigengenes, the first principal component of each module, and named by color. Module-trait associations were determined using a linear model fitted to analyze the association between the abundance profiles of the module eigengene (ME) and the phenotypic values of IMF and fatty acid profile. The list of proteins into the significant modules (p-value ≤ 0.05) that were associated with the phenotype was assigned for functional enrichment analysis. The enrichment of KEGG Pathways and Biological Processes was performed by STRING software v.11 (FDR ≤ 0.05).

Results

By applying the WGCNA framework, a total of 11 modules were identified (Figure 1). The purple module was associated with IMF and the sum of omega-3 ($p \leq 0.05$). Besides, the green-yellow module was associated with the sum of omega-3 ($p \leq 0.05$). The magenta module was positively associated with palmitic and saturated fatty acid (SFA), and negatively to oleic and MUFA ($p \leq 0.05$).

The proteins in the purple module were enriched for viral myocarditis (bta05416), tight junction (bta04530) and oxytocin signaling pathways (bta04921). Proteins in the green-yellow module were associated with striated muscle contraction (GO:0006941) and regulation of muscle contraction (GO:0006937) biological processes. The IMF development can disorganize the muscle structure, resulting in the epithelial remodeling of the extracellular matrix and cytoskeleton rearrangements [10], supporting our findings.

Proteins in the magenta module were mainly enriched for NOD-like receptor

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signaling pathway (bta04621), cholesterol metabolism (bta04979) and calcium signaling pathway (bta04020). NOD-like receptor and calcium signaling pathways have been previously pointed as involved with lipid metabolism [11, 12].

Conclusion

These results demonstrated that the tight junction pathways and muscle contraction biological process are involved with IMF deposition and omega-3 content. The NOD-like receptor and calcium signaling pathways were found linked to levels of SFA, MUFA, palmitic and oleic acids. This study provides molecular mechanisms that could influence intramuscular fat deposition and composition.

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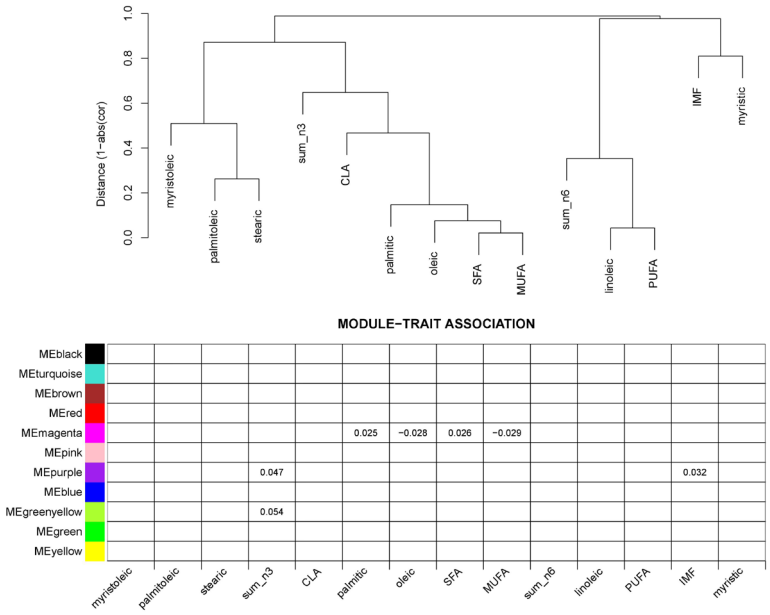
Notes



Hierarhical clustering and module-trait association analysis
Figure 1. Hierarhical clustering of phenotypic correlation between traits (top) and module-trait association analysis (botton). Modules are labeled by color on the y-axis. Each column represents a trait as indicated on the corresponding dendogram branch. For significantly associated modules ($p \leq 0.05$), the coefficient from the linear model is given within the cell.

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